

Telomerase

Telomerase Subunit

Telomerase

Telomerase Subunit

2001

12



2001 12

2

가

가

가

가

가

.....	1
•	3
•	7
1.	7
2.	7
가.	7
. MTT	8
.	8
. Senescence-associated β-galactosidase stain	9
.	9
. TRAP assay telomerase	10
. RT-PCR telomerase subunit (hTERT, hTER)	11
. Real-time PCR hTERT, c-Myc, Mad1	12
. Southern blot hybridization terminal restriction fragment (TRF)	13

•	16
1.	MTT assay AZT	16
2.	16
3.	19
4.	21
5.	TRAP assay telomerase	23
6.	RT-PCR telomerase subunit	26
7.	Real-time PCR hTEP, c-Myc, Mad1	29
8.	<i>Southern blot</i> TRF	32
9.	AZT	32
•	35
•	40
	41
	50

Figure 1. Dosage determinant by MTT assay	16
Figure 2. Growth curve of MCF-7 and MDA-MB-231	18
Figure 3. Expression of senescence by senescence associated (SA) â-galactosidase stain in breast cancer cells	20
Figure 4. Apoptosis detection by dTd enzyme in breast cancer cells	22
Figure 5. Telomerase activity of MCF-7 by TRAP assay	24
Figure 6. Telomerase activity of MDA-MB-231 by TRAP assay	25
Figure 7. Expression of hTERT and hTER mRNA of MCF-7 by RT-PCR	27
Figure 8. Expression of hTERT and hTER mRNA of MDA-MB-231 by RT-PCR	28
Figure 9. Expression of telomerase subunits mRNA by real-time PCR	31
Figure 10. Length of Terminal restriction fragments (TRFs) by Southern blot hybridization	33
Figure 11. Time points of change pattern in biological and genetic phenotype	34

Table 1. Primer oligonucleotide sequence 13

Table 2. Growth inhibition rate in MCF-7 and MDA-MB-231 17

Telomerase

Telomerase Subunit

telomere . Telomere
telomerase
telomerase 가
3' -azido-2',3' -
dideoxythymidine (AZT) telomerase telomere
telomere .
telomerase subunit telomere,
telomerase .
AZT .
X-gal 가 ,
가 . TRAP assay telomerase
가 telomerase
, telomere 가 . RT-PCR real-time PCR
telomerase subunit , hTERT가 AZT
가 가 telomerase
. hTERT c-
Myc hTERT 가 ,
hTER Mad1, hTEP가 가
. AZT telomerase subunit 가
hTERT c-Myc 가 , telomerase
subunit 가 telomerase telomere

가

.

: Telomerase, Telomere, , ,
hTERT, hTER, hTEP, c-Myc, Mad1.

Telomerase

Telomere Subunit

<

>

(senescence)

telomere

가

telomere

가

.

가

가

'mitotic clock'

DNA

telomere

(TTAGGG)_n

.² Telomere
 DNA
 ,³⁻¹⁰ DNA polymerase
 'end-replication problem' 가 telomere
 50-200 nucleotide가 .^{11,12} telomere
 ,
 가 .¹³⁻¹⁵
 Telomere 가 .
 duplex telomere TTAGGG-repeat binding factor TRF1¹⁶
 TRF2⁵가 telomere
 telomere . Single strand telomere
 telomerase reverse transcriptase (TERT),¹⁷ telomere associated protein
 1 (TEP1),^{18,19} hnRNP A1²⁰ telomere telomere
 telomere .
 Telomere telomerase 가 telomerase
²¹
 RNA 가
 telomere TTAGGG telomere
 . Telomerase telomere 가
 RNA (TER) catalytic 가 TERT .
 hTER telomere
 telomere template domain pseudoknot structure,
 CR4-CR5 domain, H/ACA box CR7 domain .^{22,23}
 telomerase hTER
 telomerase associated protein 1 (TEP1)
 가 hTERT가 .^{24,25} hTERT 1132
 hTERT T-motif
 6 motif
 telomerase . hTERT hTERT
 promoter
 alternative splicing,²⁶ hTERT
 . hTERT

가 c-Myc²⁷ Mad²⁸가 .
 hTERT mRNA alternative splicing domain A
 12 hTERT α, domain A domain B
 182 가 hTERT β, α
 β가 가 .²⁶ protein kinase
 hTERT telomerase
 .²⁹
 telomere가 ‘Hayflick
 limit’³⁰ ,
 (transformation) .
 telomerase가 crisis
 telomerase가 (immortalization) .
 telomerase ,^{31,32} telomere
 .³³⁻³⁶
 Telomerase 31
 가 . telomerase
 가 telomerase
 가 .
 , hTER hTER anti-sense nucleotide
 ,³⁷⁻³⁹ hammerhead ribozyme , catalytic component
 hTERT .
 Telomerase RNA
 (reverse transcriptase, RT) hTERT
 telomerase 가 . Dideoxyguanosine (ddG),
 dideoxyinosine (ddI), 3’-azido-2’,3’-dideoxythymidine (AZT), AZT-5’
 triphosphate (AZT-TP) retrovirus
 .^{40,41} AZT ADIS
 AZT가 thymidine kinase AZT-MP ,

thymidylate kinase AZT-DP , nucleoside diphosphate
kinase AZT-TP ,⁴² AZT-TP

가 DNA . AZT-MP DNA
DNA template primer

⁴³⁻⁴⁶

AZT

가

⁴⁷

⁴⁸

AZT

^{49,50}

AZT

51

, AZT

telomeric

DNA

telomerase

telomere

^{40,52-56} *in*

vitro

AZT

telomerase

.

telomerase subunit

telomere, telomerase

telomerase

.

1.

MCF-7 (ATCC HTB 22) MDA-MB-231 (ATCC HTB 26)
Primer oligonucleotide GENSET (Singapore Biotech Pte Ltd, Singapore), Amersham Pharmacia (Piscataway, NJ, USA), Sigma (St Louis, MO, USA)

2.

가.

MCF-7 MDA-MB-231 minimum essential medium (MEM, GIBCO BRL, Grand Island, NY, USA) 10% (FBS, GIBCO BRL, Grand Island, NY, USA) penicillin 100 unit/ml streptomycin 100 µg/ml (GIBCO BRL, Grand Island, NY, USA) 가
37°C, 5% CO₂ 2-4
hemocytometer 5
3 passage 0.25% Trypsin-EDTA (GIBCO BRL, Grand Island, NY, USA)
-70 population doubling (PD)

$2^{PD} = (\text{dilution factor})$

$\log 2^{PD} = \log (\text{dilution factor})$

$$PD = \log(\text{dilution factor}) / \log 2 = \log 5 / \log 2 = 2.32$$

$$PD = 2.32 \times (\text{passage number})$$

. **MTT**

MTT

.⁵⁷

MCF-7 MDA-MB-231 0.25% Trypsin-EDTA
 , 10% FBS가 MEM
 . Trypan blue , 180 $\mu\ell$
 5 x 10³ 96-well plate .
 37°C, 5% CO₂ 16 AZT
 ,
 가 . Plate 4 2 mg/ML MTT 50 $\mu\ell$
 well 가 4 .
 plate 450 x g 10 . formazan
 150 $\mu\ell$ dimethyl
 sulfoxide (DMSO) 가 37°C 10 formazan
 . ELISA reader 540nm

.⁵⁸

inhibitory concentration, 10% (IC₁₀), IC₂₀

가 .

$$(\%) = \frac{\text{---}}{\text{---}} \times 100$$

5 x 10⁴

culture plate . 24 6
 hemocytometer .
 3 well .
 log phage
 가 (doubling
 time, DT) .⁵⁹

. **Senescence-associated β -galactosidase stain**

β -galactosidase .⁶⁰
 24-well plate 5 x 10⁴ 16 37 °C, 5% CO₂
 . 가 plate phosphate buffered-
 saline(PBS) 2% formaldehyde / 0.2% glutaraldehyde (
 3 % formaldehyde) 5 . PBS
 senescence-associated β -galactosidase stain [40 mM citric
 acid/sodium phosphate buffer (pH6.0), 150 mM NaCl, 5 mM potassium
 ferrocyanide, 5 mM potassium ferricyanide, 2 mM MgCl₂] 20 mg/M ℓ X-
 Gal mg/M ℓ 가 37 16
 . PBS
 . 100
 5

DNA

Apoptaq Peroxidase *In Situ* Apoptosis Detection Kit (Intergen company,
 Purchase, NY, USA) . 1 x 10⁴
 96-well plate 16 . PBS

1% paraformaldehyde 10 ethanol : acetic acid
 2:1 5 -20 . Kit dTd
 37 , 1 .
 Peroxidase substrate diaminobenzidine (DAB, DAKO Corporation,
 Carpinteria, CA, USA) 30 0.5% methyl green
 15
 . 100
 5

TRAP assay telomerase

Telomerase telomeric repeat-amplification protocol (TRAP) assay
 .⁶¹ PCR
 . telomerase substrate TS
 oligonucleotide primer가 telomerase TTAGGG
 ACX primer가 reverse primer
 PCR . Telomerase
 telomerase 293 (ATCC
 CRL 1573) . CHAPS lysis
 buffer TRAP
 . Taq polymerase 가
 PCR 가 ^{62,63} 36
 internal control primer PCR 가 .
 PBS 1 M TRAP [10 mM HEPES-KOH
 (pH7.5), 1.5 mM MgCl₂, 10 mM KCl, 1 mM dithiothreitol] 10000 x g,
 4 1 . 가 가 lysis
 buffer [10 mM Tris-HCl (pH7.5), 1 mM MgCl₂, 1 mM EGTA, 0.1 mM PMSF, 5
 mM β-mercaptoethanol, 10% glycerol] 50 μℓ 30
 . 14000 x g 4 30
 Bradford method

inhibitor, 40 units M-MLV reverse transcriptase가 [250
 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM MgCl₂, 50 mM DTT]
 20 μℓ가 37 1 70
 10 가 cDNA . cDNA 5 μℓ 1.5 μCi [α-
³²P]dCTP, 5 mM dNTP, 1.5 units Taq polymerase PCR
 hTERT PCR 5 pM primer TERT 1784S, TERT 1928A
 house-keeping gene â-actin
 . Internal control primer 774, 775 2.5 pM 25 μℓ PCR
 . 94 3
 94 30 , 60 30 , 72 30 30 hTER PCR
 2.5 pM primer TR-46S, TR-148A 5 pM â-actin internal control primer
 5899, 5900 가 94 3 94
 30 , 60 30 , 72 30 22 PCR
 PCR 5% polyacrylamide gel
 autoradiography .⁶⁴ sample â-actin internal
 control PCR band intensity

Real-time PCR hTERT, c-Myc, Mad1

cDNA telomerase subunits
 real-time PCR
 . hTERT, hTER,
 hTERT, c-Myc, Mad1 primer Table 1 .
 cDNA QuantiTect SYBR Green PCR Kit (QIAGEN, Santa Clarita,
 CA, USA) . Kit HotstarTaq DNA polymerase,
 QuantiTect SYBR Green PCR buffer, dNTP mix including dUTP, SYBR Green
 , ROX (passive reference dye), 5 mM MgCl₂가
 QuantiTect SYBR Green PCR Master Mix 가 PCR
 primer 0.5 μM 가 20 μℓ PCR

. Rotor-Gene 2072D (Corbett Research, Australia)

95 15 94 15 , 60 20 , 72
20 45 PCR .

Table 1. Primer oligonucleotide sequence

Primer	
TS	5' -AATCCGTCGAGCAGAGTT-3'
ACX	5' -GCGGCGGCTTACCCTTACCCTTACCCTA-3'
TSNT	5' -AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3'
NT	5' -ATCGCTTCTCGGCCTTTT-3'
R8	5' -AATCCGTCGAGCAGAGTTAG[GGTTAG] ₇ -3'
TERT 1784S	5' -CGGAAGAGTGCTCTGGAGCAA-3'
TERT 1928A	5' -GGATGAAGCGGAGTCTGGA-3'
â-actin 774	5' -GGGAATTCAAACCTGGAACGGTGAAGG-3'
â-actin 775	5' -GGAAGCTTATCAAAGTCCTCGGCCACA-3'
TR-46S	5' -CTAACCCTAACTGAGAAGGGCGTAG-3'
TR-148A	5' -GAAGGCGGCAGGCCGAGGCTTTTCC-3'
â-actin 5899	5' -CAGGTCATCACCATTGGCAATGAGC-3'
â-actin 5900	5' -CGGATGTCCACGTCACACTTCATGA-3'
c-Myc-S	5' -AAGTCCTGCGCCTCGCAA-3'
c-Myc-AS	5' -GCCTGTGGCCTCCAGCAGA-3'
Mad1-S	5' -TTCAGACTTGGACTGTGTCA-3'
Mad1-AS	5' -ACGCTGAGAGATGAAGTTGT-3'
hTEP-S	5' -TCAAGCCAAACCTGAATCTGAG-3'
hTEP-AS	5' -CCCGAGTGAATCTTTCTACGC-3'

. Southern blot hybridization terminal restriction fragment (TRF)

300 $\mu\text{g}/\text{Ml}$ Proteinase K 50 $\mu\text{g}/\text{Ml}$ RNase A가 DNA
 lysis buffer , phenol/chloroform ethanol
 DNA . 10 μg DNA Hinf
 (Promega, Madison, WI, USA) 15 unit DNA .
 0.5 $\mu\text{g}/\text{Ml}$ ethidium bromide (EtBr)가 0.8% agarose gel
 DNA gel loading buffer
 . UV-transilluminator gel
 DNA size marker size . Gel
 EtBr gel loading buffer
 (0.5 M NaOH, 1.5 M NaCl) gel 30
 가 2 . gel
 (1.5 M NaCl, 0.5 M Tris-Cl, pH 8.0)
 30 . Gel DNA
 Hybond-N membrane (Amersham Pharmacia, Piscataway, NJ, USA)
 transfer capillary transfer 10X SSC (1.5
 M NaCl, 0.15 M sodium citrate) 16 DNA transfer
 . Transfer가 UV transilluminater transfer가
 membrane DNA가
 UV cross linking . membrane 42 1
 pre-hybridization . Pre-hybridization
 lable oligonucleotide probe 가 42 16
 hybridization . Pre-hybridization Rapid-Hyb buffer (Amersham
 Pharmacia, Piscataway, NJ, USA) hybridization
 . Probe telomere 5' -[TTAGGG]₆-3'
 [α -³²P]ATP(3000 Ci/mM) end-lable . 30 pM
 [TTAGGG]₆ 50 pM [α -³²P]ATP 20 unit T4 polynucleotide kinase
 (New England Biolabs, Beverly, MA, USA) 37 1
 . 0.1 mM EDTA(pH 8.0)
 MicroSpinTM G-25 column (Amersham Pharmacia, Piscataway, NJ, USA)
 spin column chromatography

	Hybridization	membrane	1	(2X SSC,
0.2% SDS)	15	가		2
2	(0.1X SSC, 0.1% SDS)		42	10
0.1X SSC		autoradiography		TRF

. ¹¹

$$\text{TRF}(\text{mean}) = (\text{OD}_i \times L_i) / (\text{OD}_i)$$

OD_i : signal intensity over interval i

L_i : kilobase size at the middle of interval i

1. MTT assay AZT

AZT MTT assay
 AZT 10% (10%
 growth inhibitory concentration, IC₁₀) 20% (IC₂₀)
 AZT(low dose; DL) MCF-7 20 μM, MDA-MB-231 25
 μM
 AZT(high dose; DH) MCF-7 70 μM,
 MDA-MB231 50 μM (Fig.1).
 (CT)

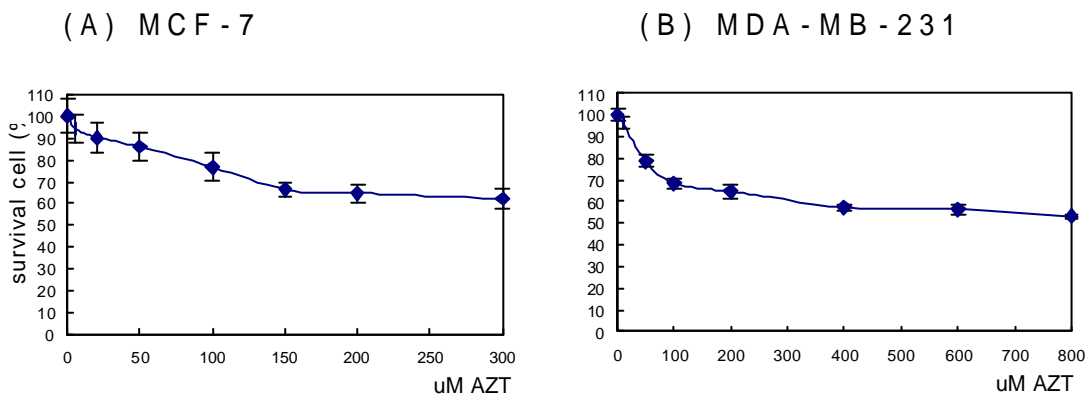


Figure 1. Dosage determinant by MTT assay. (A) MCF-7, (B) MDA-MB-231.

2.

AZT

MCF-7 PD가 7PD가 , 53PD가
high dose low dose
(60PD)

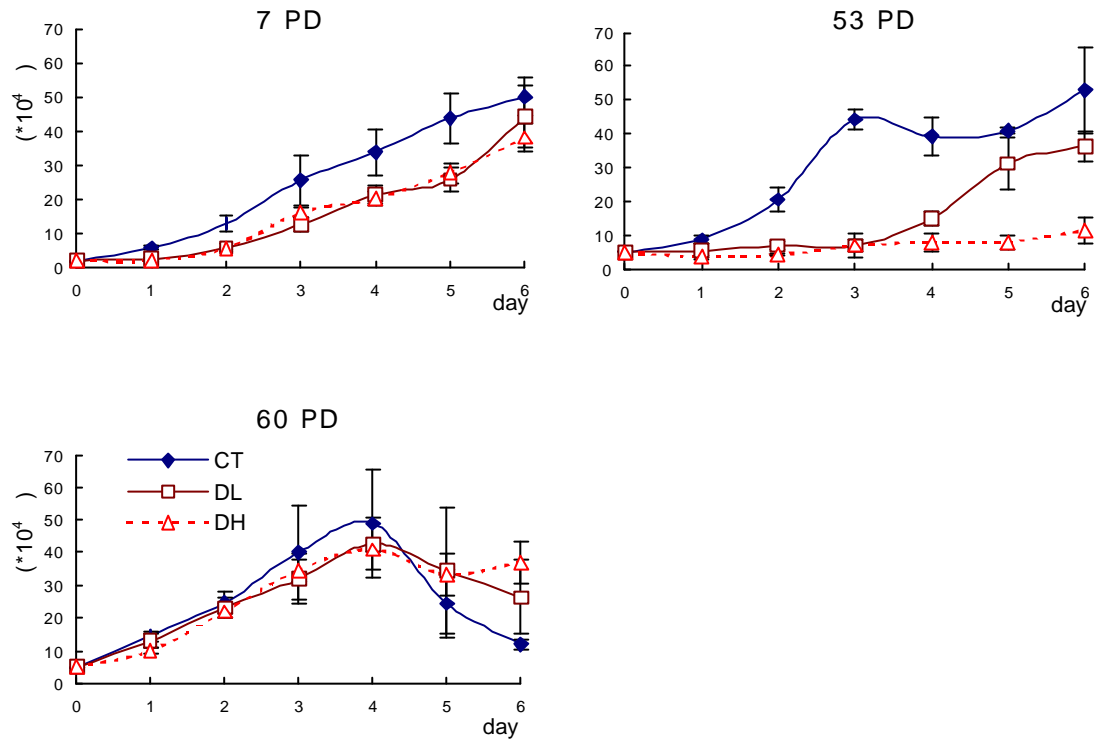
MDA-MB-231 PD AZT 90PD 42PD low dose high dose
50%, 66% (Fig. 2).
(Table 2).

Table 2. Growth inhibition rate in MCF-7 and MDA-MB-231

(A) MCF - 7				(B) MDA - MB - 231		
PD	7PD	53PD	60PD	PD	42PD	90PD
AZT	+	+	-	DL	25.1%	49.7%
DL	41.2%	61.9%	12.9%	DH	33.3%	65.8%
DH	36.9%	79.9%	16.3%			

+: AZT treatment, -: removal of AZT.

(A) MCF - 7



(B) MDA - MB - 231

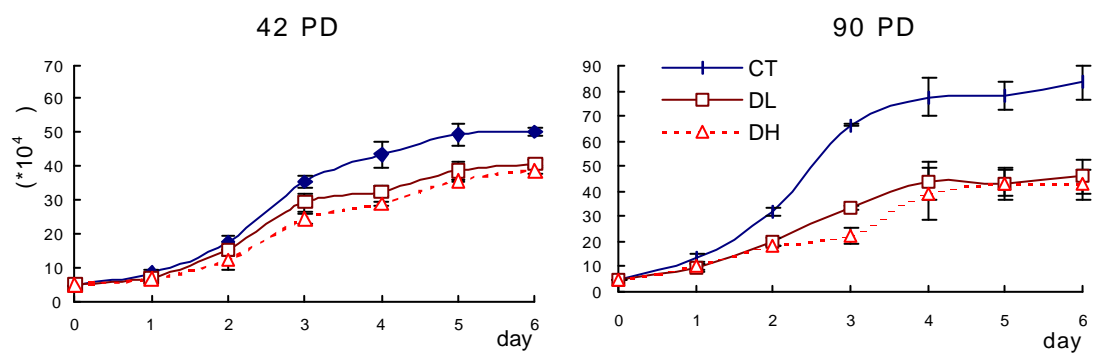


Figure 2. Growth curve of MCF-7 and MDA-MB-231. (A) MCF-7, (B) MDA-MB-231. CT(): control, DL(): low dose, DH():high dose, PD: population doubling.

3.

AZT telomerase X-gal

MCF-7

가 가 low dose high dose PD가 가

가 가 가 가 PD

가 가 30PD 가

(28PD: 2%, low dose 5%, high dose 13%). 53PD

가 low dose 12%, high dose 15% 2.8%

가 4, 5 가 가 AZT AZT

가

77PD low dose , high dose

MDA-MB-231 가

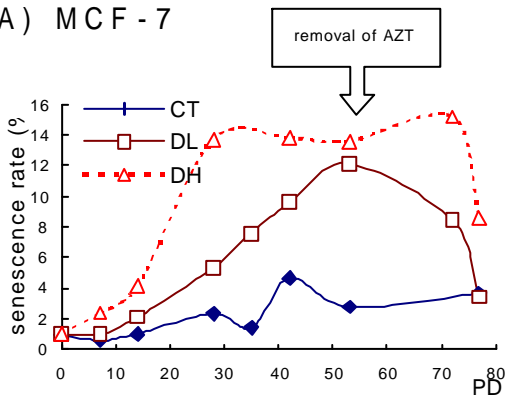
42PD 2% low dose, high dose

5.4%, 5.8% 2.5 가 56PD 10 가

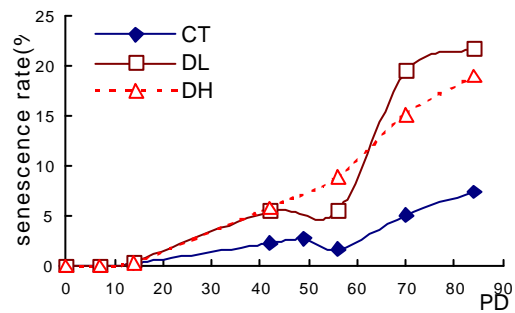
PD가 가 가

가 가 (Fig. 3).

(A) MCF-7



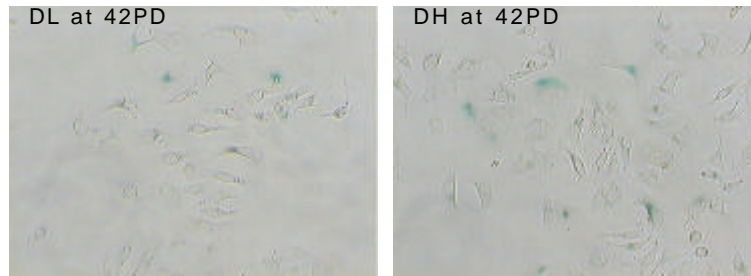
(B) MDA-MB-231



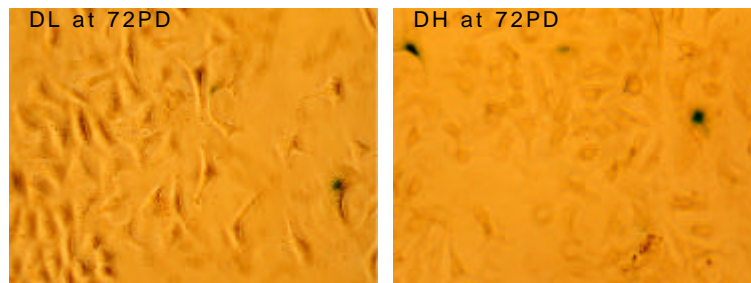
(C) MCF - 7

AZT

(+)



(-)



(D) MDA - MB - 231

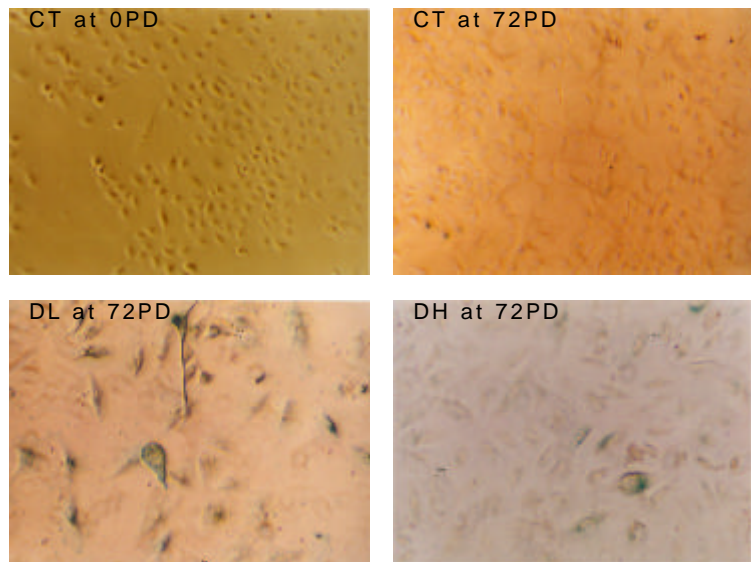
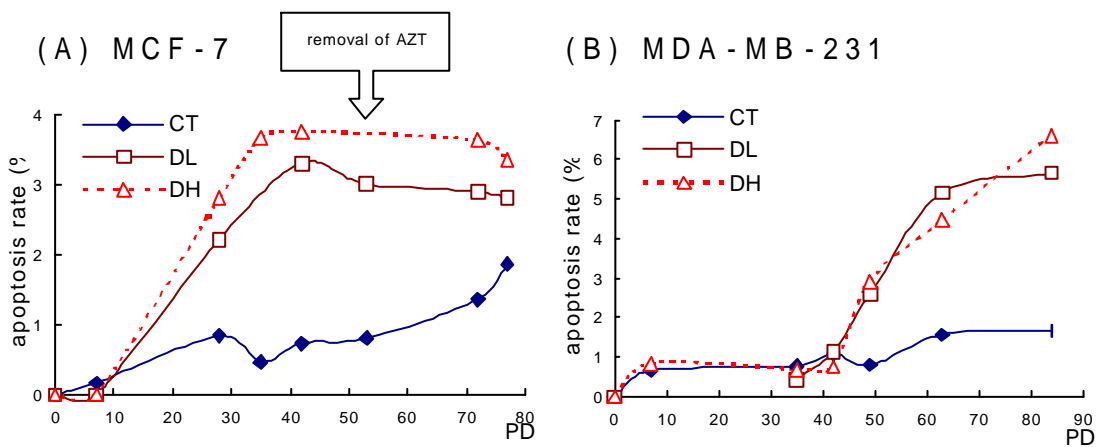


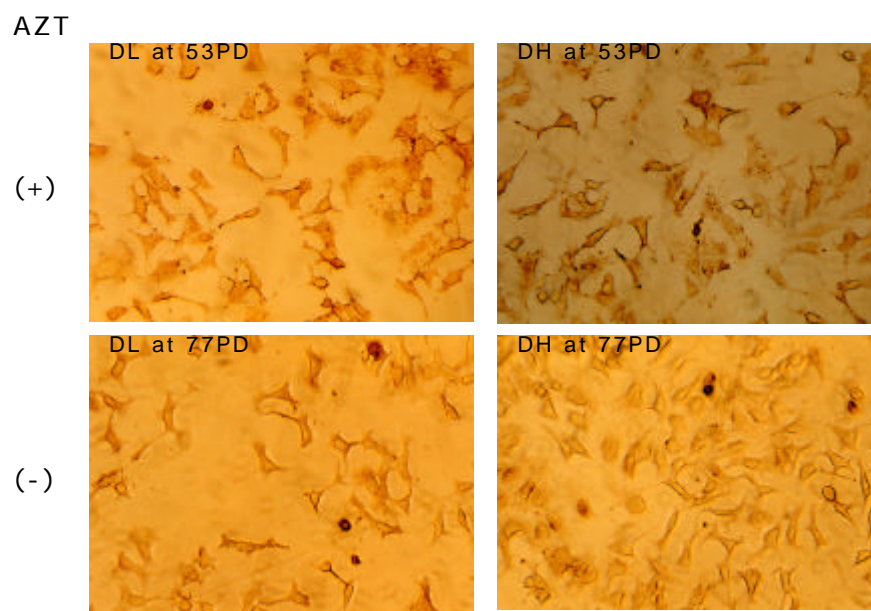
Figure 3. Expression of senescence by SA β -gal stain in breast cancer cells. (A,C) MCF-7, (B,D) MDA-MB-231. CT(): control, DL(): low dose, DH(): high dose, PD: population doubling, +: AZT treatment, -: removal of AZT.

4.

AZT 가
dTd apoptotic-body .
MCF-7 , PD 가 1%
PD가 가 low dose
28PD 2.2% PD (0.8%) 3
가 high dose(2.8%) 가 PD가
가 , 42PD (0.7%)
low dose(3%), high dose(3.7%)
. AZT
. .
MDA-MB-231 , PD 가
가, 50PD low dose, high dose 가
. 49PD (0.8%) low dose(2.6%), high dose(2.9%)
3 가 , PD가 가
가 (Fig. 4).



(C) MCF - 7



(D) MDA - MB - 231

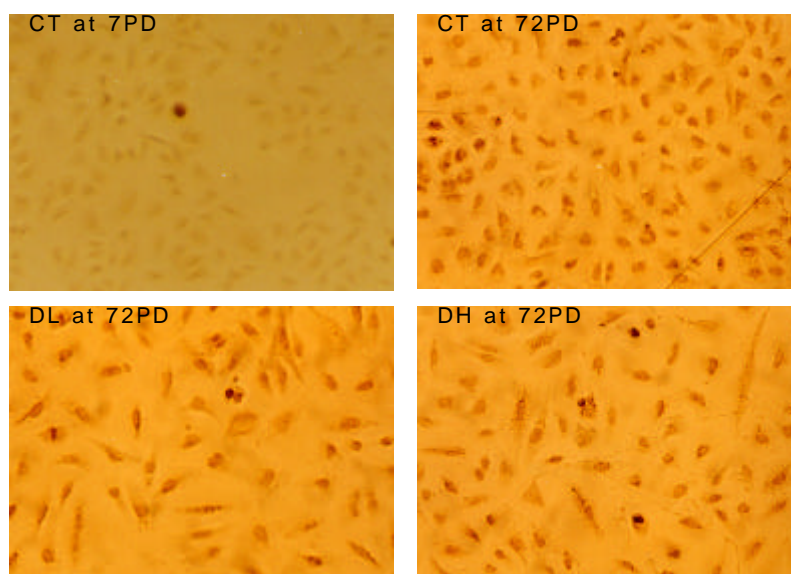


Figure 4. Apoptosis detection by dTd enzyme in breast cancer cells. (A,C) MCF-7, (B,D) MDA-MB-231. CT(): control, DL(): low dose, DH(): high dose, PD: population doubling, +: AZT treatment, -: removal of AZT.

5. TRAP assay telomerase

Telomerase TRAP assay .
 293 telomerase
 , lysis buffer PCR
 TRAP band IC band quality
 control .
 TRAP assay , PD가 가 telomerase가
 , AZT telomerase
 . MCF-7 , low dose high dose 가
 PD 42PD 53PD
 . AZT telomerase 가
 가 , AZT low dose high dose
 telomerase 가 (Fig. 5).
 MDA-MB-231 low dose, high dose telomerase
 가 MCF-7 . low dose, high dose
 42PD . Telomerase
 PD가 가 MCF-7
 (Fig. 6). MDA-
 MB-231 MCF-7 telomerase 가 MCF-7
 AZT telomerase 가 .

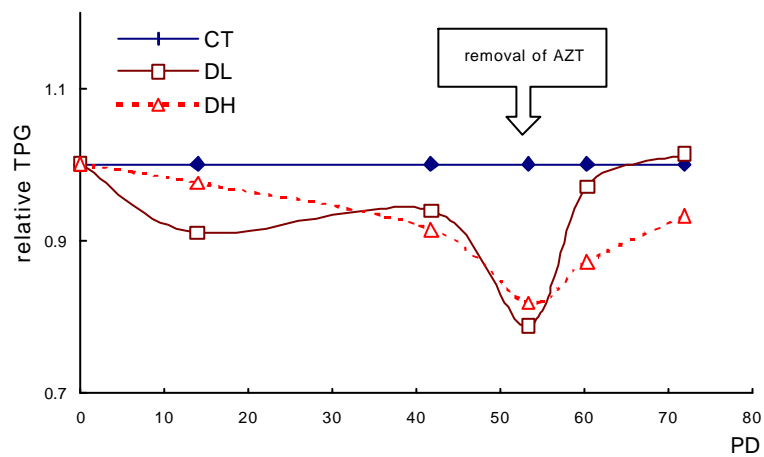
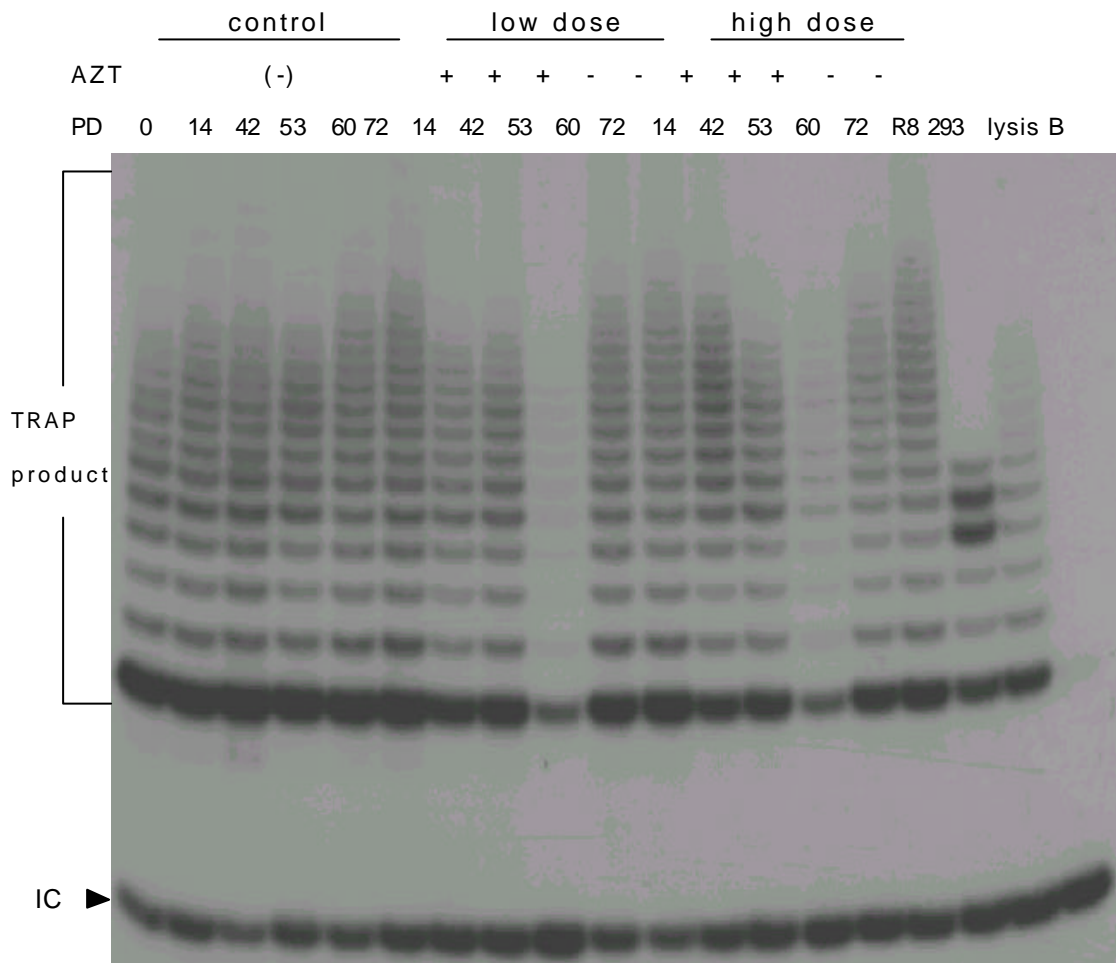


Figure 5. Telomerase activity of MCF-7 by TRAP assay. IC: internal control, R8: R8 control template, 293: 293 cell, lysis B: lysis buffer, +: AZT treatment, -: removal of AZT.

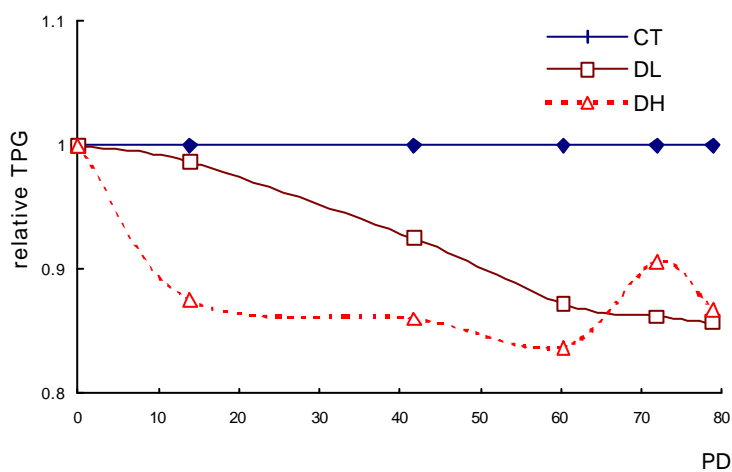
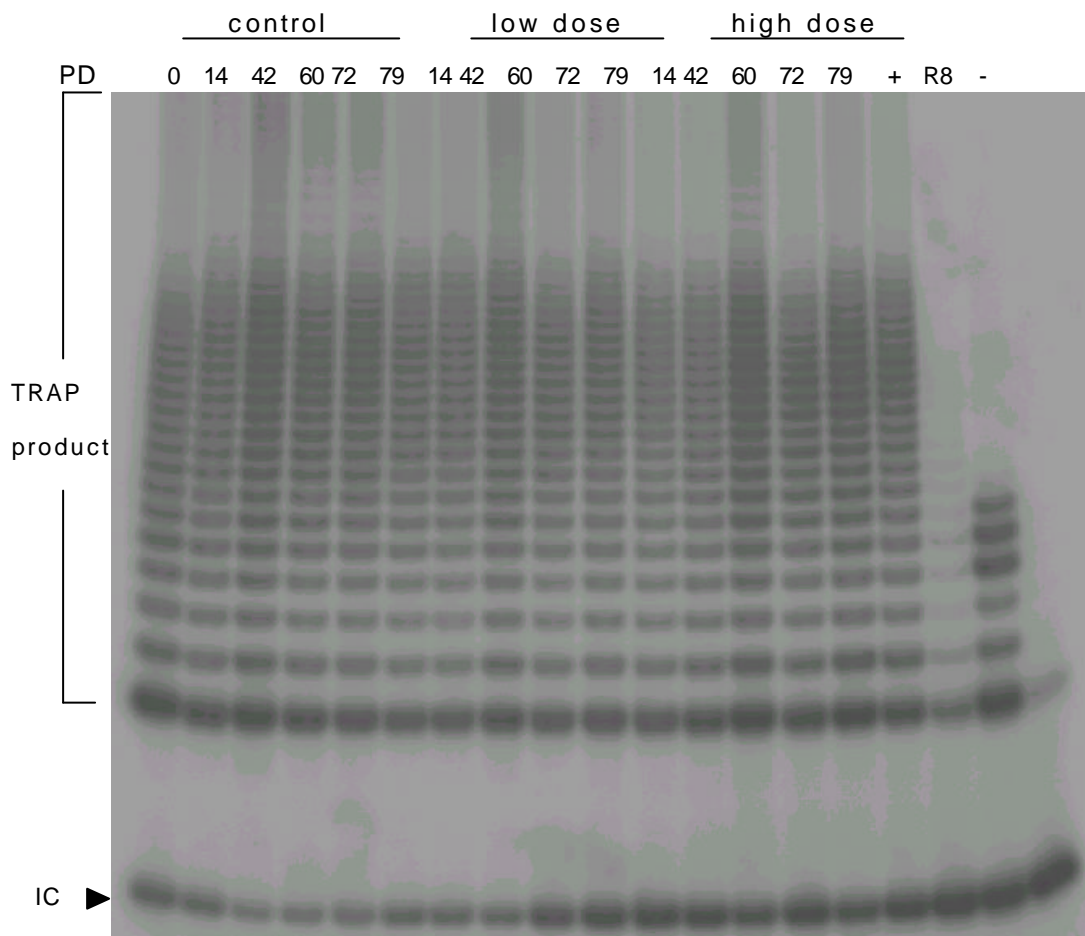


Figure 6. Telomerase activity of MDA-MB-231 by TRAP assay. IC: internal control, R8: R8 control template, +: 293 cell, -: lysis buffer.

6. RT-PCR telomerase subunits

Telomerase, hTERT, hTER, RT-PCR, α -actin primer

telomerase

hTERT, AZT, MCF-7

low dose 10PD, hTERT가, PD가 가

. High dose

hTERT 가, 53PD, AZT 가 .

hTER, hTERT, MCF-7, low dose, 28PD 가, AZT

. High dose

(Fig.7).

MDA-MB-231, hTERT, low dose, 20PD, PD가 가

. High dose, PD, hTERT

PD가 . hTER

low dose, high dose, 42PD

hTER (Fig.8).

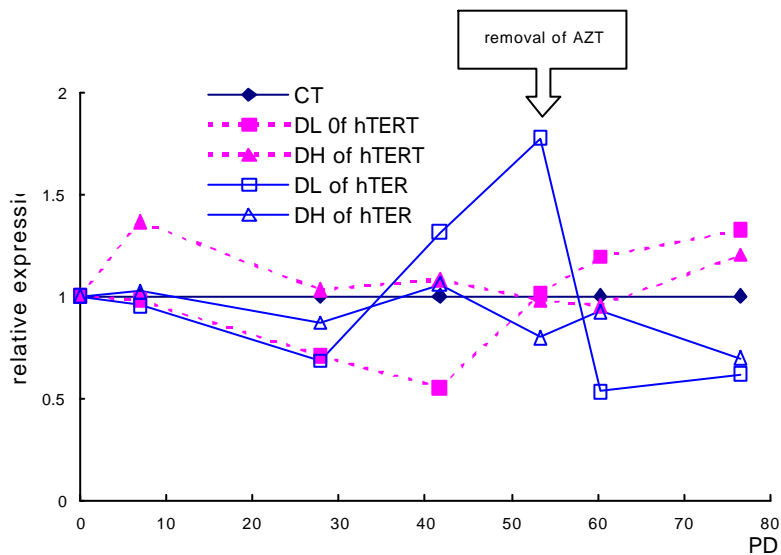
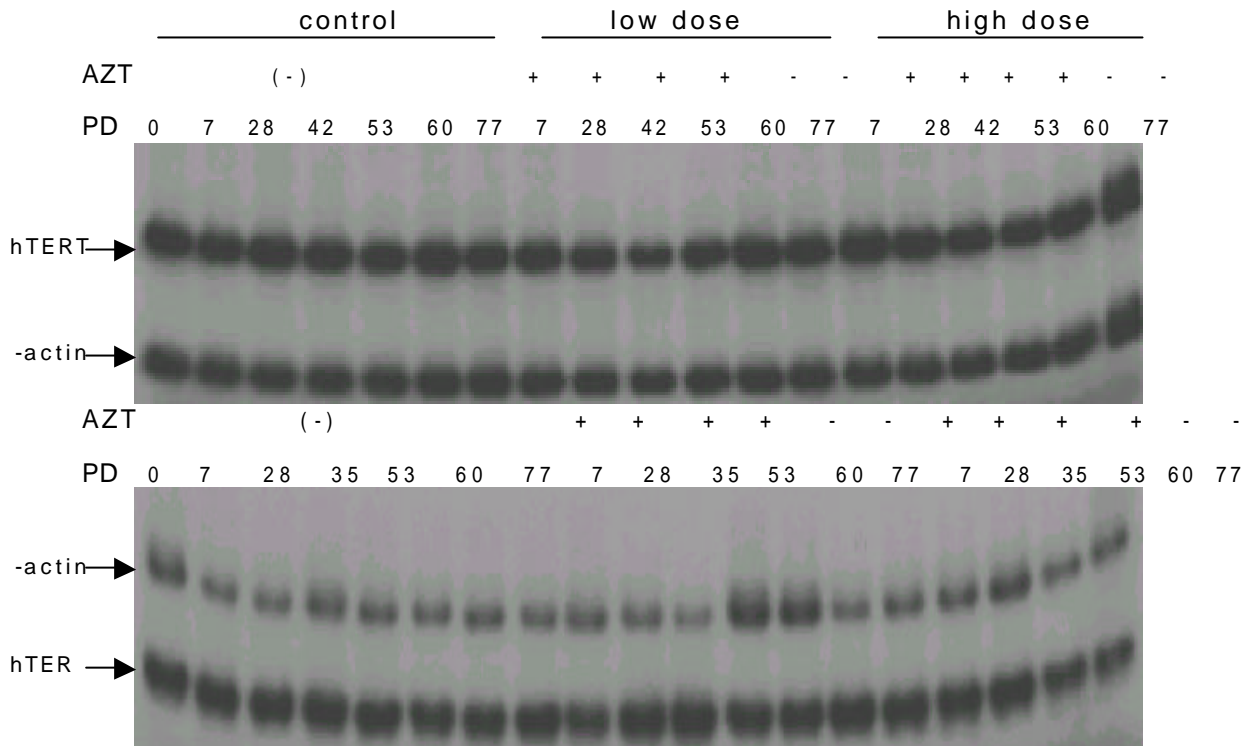


Figure 7. Expression of hTERT and hTER mRNA of MCF-7 by RT-PCR. CT(): control, DL(): low dose, DH(): high dose, +: AZT treatment, -: removal of AZT.

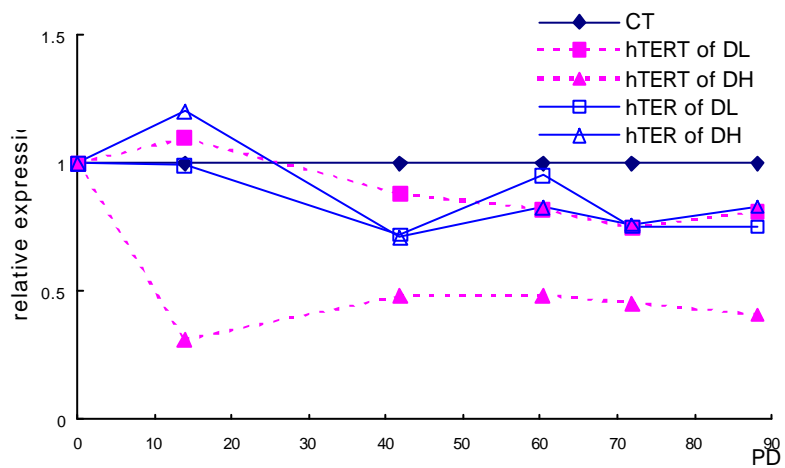
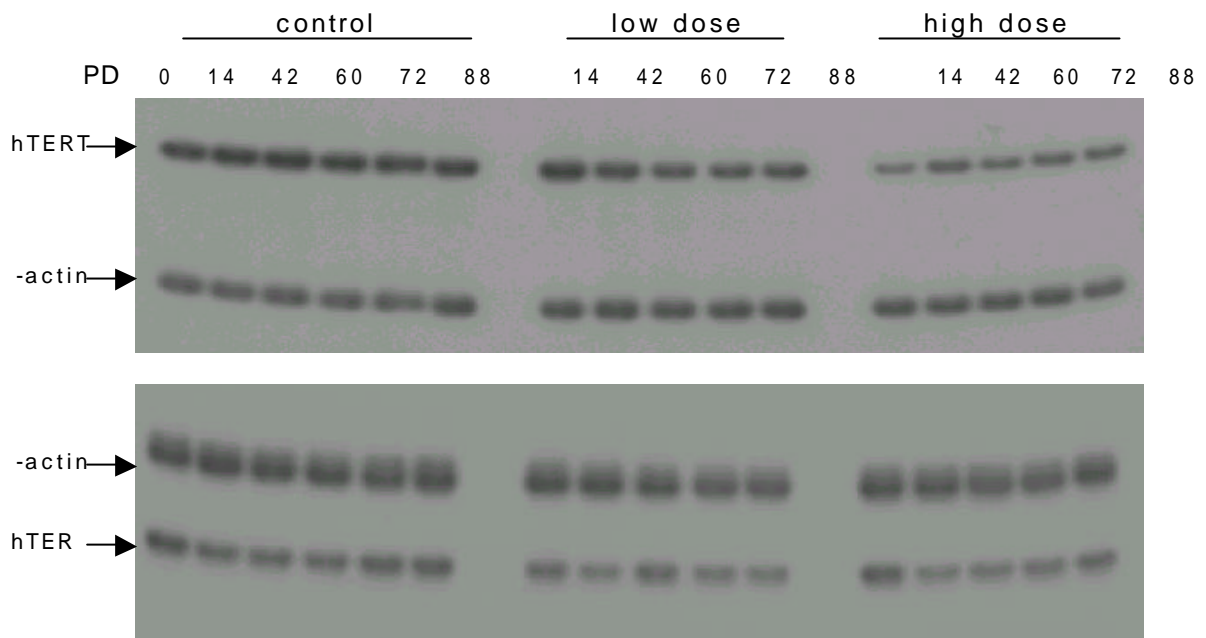


Figure 8. Expression of hTERT and hTER mRNA of MDA-MB-231 by RT-PCR. CT(): control, DL(): low dose, DH(): high dose.

7. Real-time PCR hTEP, c-Myc, Mad1

Real-time PCR telomerase RNA
 . RT-PCR hTERT, hTER
 real-time PCR RT-PCR 가
 . real-time PCR RT-PCR
 가 RT-PCR

MCF-7 low dose hTERT hTER RT-PCR
 . c-Myc AZT
 hTERT 가 Mad1
 PD c-Myc 가 가
 , AZT c-Myc
 . hTEP PD 가 , 40PD
 가 high dose RT-PCR 가
 hTERT hTER 가
 , hTERT 가 AZT
 . hTER hTERT
 hTERT
 . c-Myc 가 가

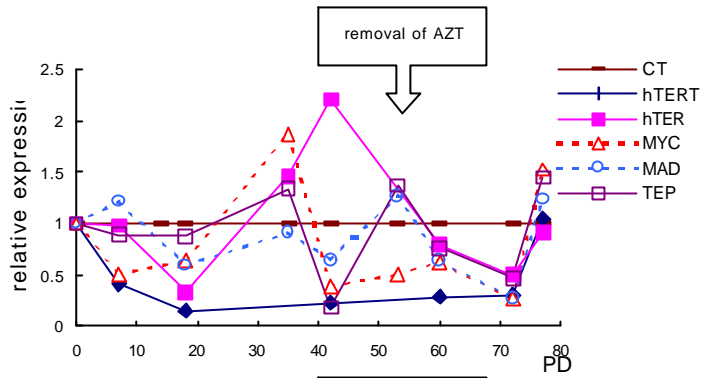
low dose c-Myc . Mad1 hTERT
 가 가 c-Myc Mad1
 가 . hTEP low dose 가
 PD가 가

MCF-7 low dose high dose hTERT
 가 c-Myc 가
 . hTER 가 , hTEP가 가
 . Mad1 hTERT c-Myc

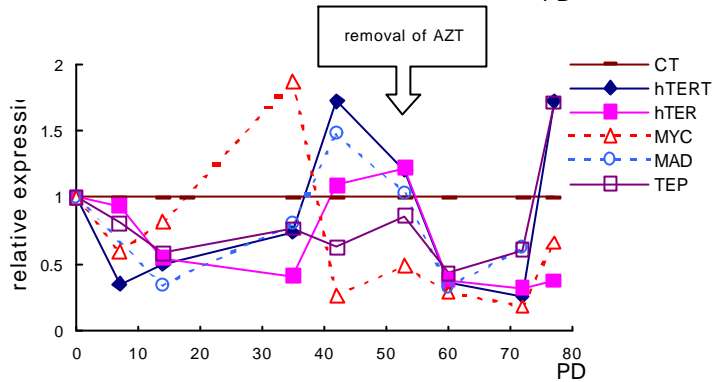
MDA-MB-231 low dose hTERT 가

가 , hTERT 20PD ,
 PD가 가 , c-Myc
 hTERT , hTERT
 . Mad1 가 , hTEP PD
 PD
 .
 High dose low dose
 . hTERT가 가 , hTER
 Mad1, hTEP 10PD
 . c-Myc hTERT
 .
 MDA-MB-231 hTERT가 가
 가 , 가
 가 , c-Myc hTER, Mad, hTEP
 가 (Fig. 9).

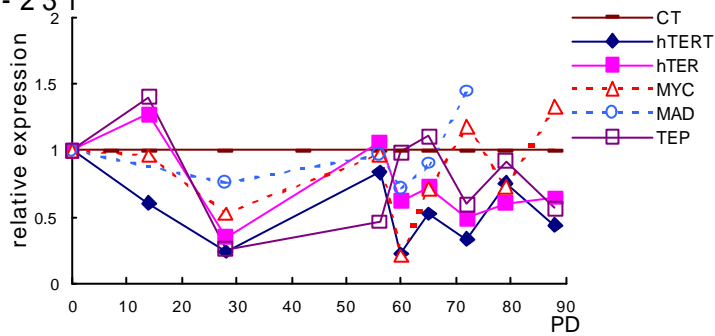
(A) MCF-7
low dose



(B) MCF-7
high dose



(C) MDA-MB-231
low dose



(D) MDA-MB-231
high dose

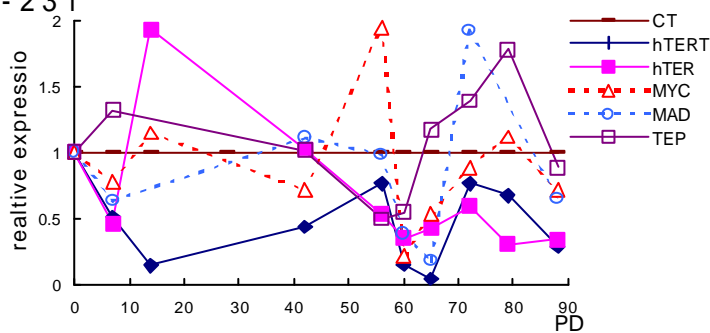


Figure 9. Expression of telomerase subunits mRNA by real-time PCR. (A)(B) MCF-7, (C)(D) MDA-MB-231. PD: population doubling, - : control, : hTERT, : hTER, : c-Myc, : Mad1, : hTEP.

8. Southern Blot

TRF

Telomere
MCF-7 PD가 가
가 low dose high dose
. telomere 가
. AZT
. MDA-MB-231
telomere low dose high dose 42PD
. PD가 가

(Fig. 10).

9. AZT

AZT
(, ,)
(telomerase , telomerase subunits, telomere)
(Fig. 11).

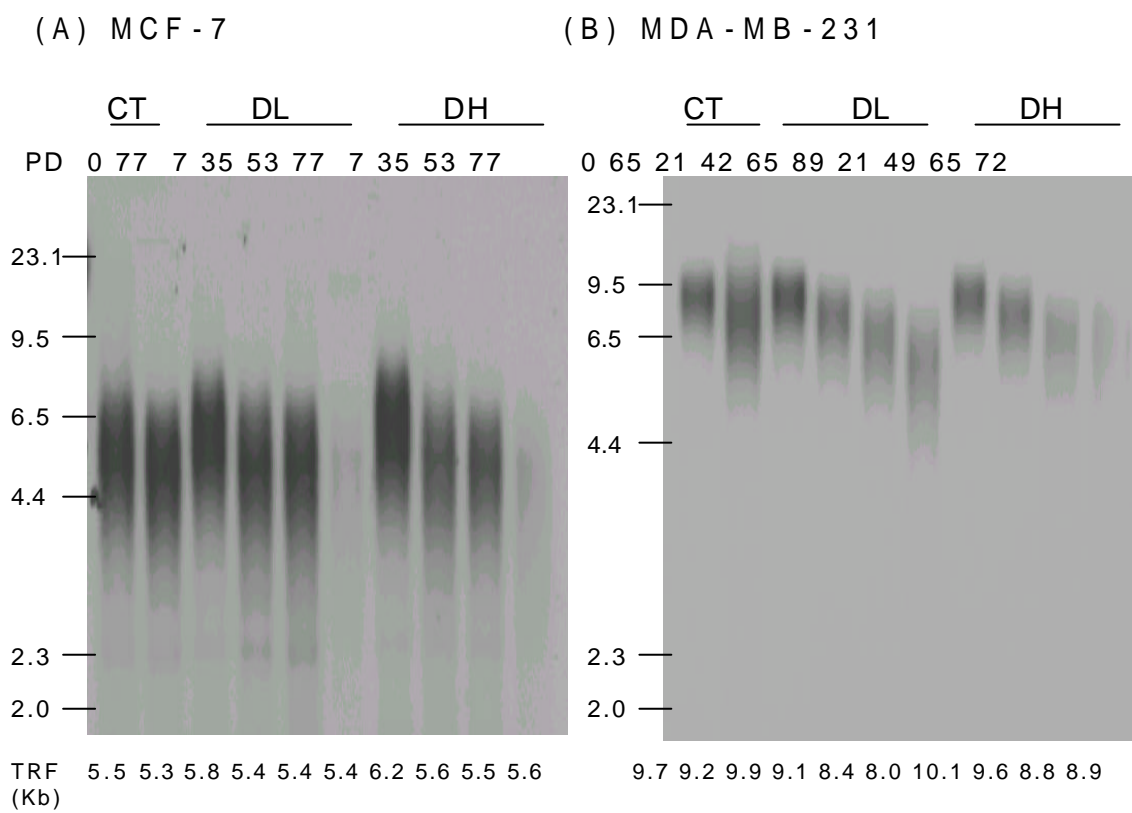


Figure 10. Length of terminal restriction fragments (TRFs) by *Southern* blot hybridization. (A) MCF-7, (B) MDA-MB-231. CT: control, DL: low dose, DH: high dose.

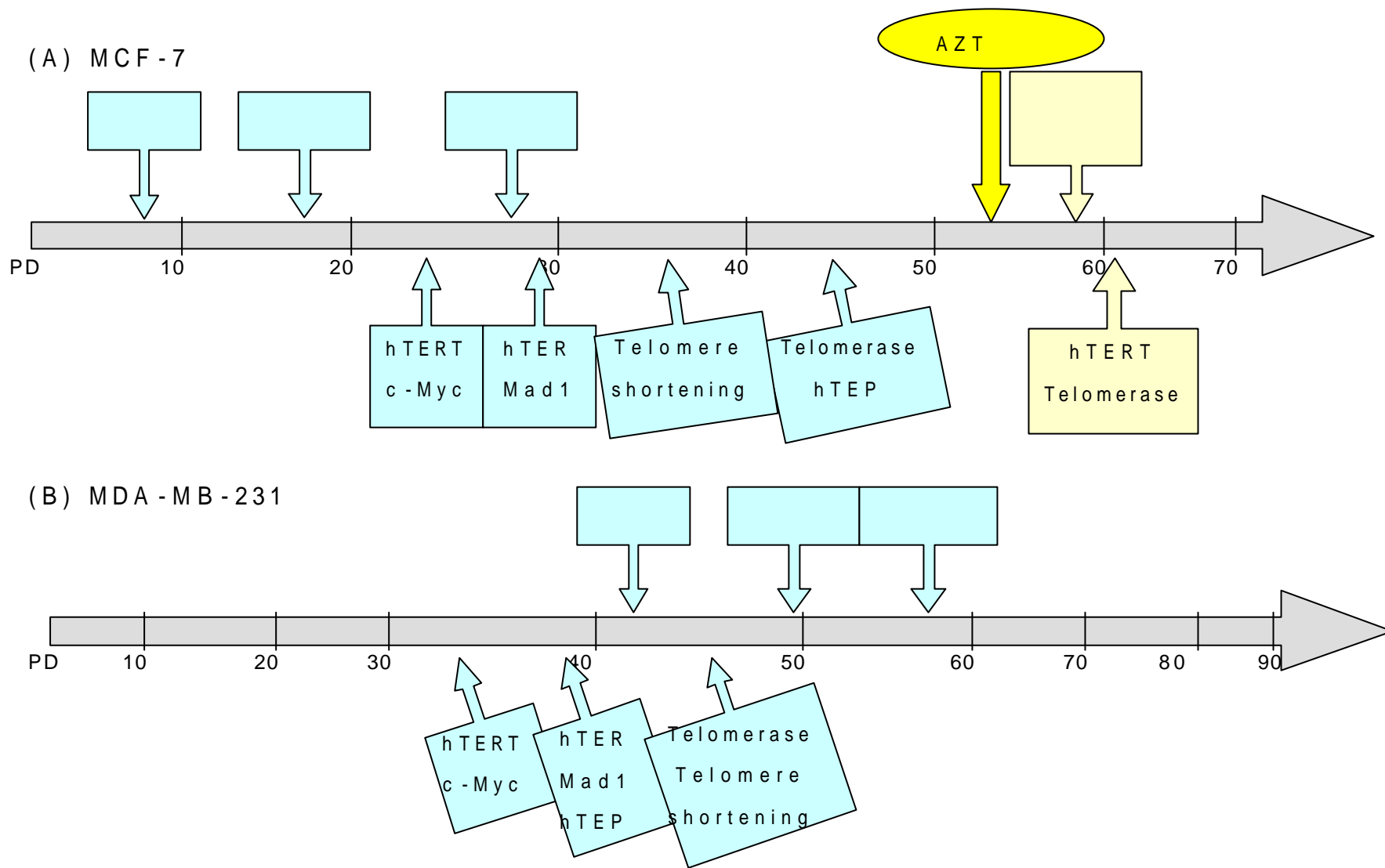


Figure 11. Time points of change pattern in biological and genetic phenotype. (A) MCF-7, (B) MDA-MB-231. Remove the AZT at 53PD of low dose and high dose cells in MCF-7.

.
 telomere 가
 가
 telomerase subunits 가 .
 AZT ADIS
 telomere . 42-46
 . 47,48
 IC₁₀, IC₂₀ AZT
 MTT assay
 AZT
 AZT
 가 ,
 가 가 ,
 가 가
 AZT
 가 AZT
 AZT 가 ,
 가
 AZT가 가 가 .
 telomere 가
 G₀
 가 AZT가
 telomere 가 DNA
 DNA
 DNA
 cell cycle

DNA가
 가
 가
 telomerase가
 telomere
 telomerase
 telomerase
 AZT
 가
 AZT
 AZT
 DNA
 PD가
 가
 AZT telomerase telomere
 가
⁶⁵⁻⁶⁸ AZT
 가
 가
 AZT가
 G₀
 AZT
 TRAP assay telomerase , PD가 가
 AZT telomerase 가
 AZT 가 가 가
 telomerase 가 AZT
 AZT가
 telomerase

telomerase subunit
 hTERT, hTER, hTEP telomerase
 . hTERT PD telomerase
 . Telomerase
 hTERT 가 PD가
 , telomerase 가 hTERT
 . AZT
 hTERT 가 , telomerase
 가 가
 telomerase hTERT ,
 telomerase hTERT ,
 hTERT 가 telomerase
 .
 hTERT가 AZT hTER
 hTERT hTER AZT
 . hTERT
 . AZT 가 hTER
 hTERT .
 hTER hTERT telomerase
 .^{22,69} hTER
 가 telomerase
 , telomerase .^{70,71}
 hTERT가 telomerase 가
^{6,72-74}
^{26,29,75,76} hTERT telomerase 가
 hTERT 가 hTER telomerase
 telomerase , hTER telomerase
 . , AZT hTERT가
 hTERT
 hTER hTER hTERT

가
 telomerase subunits telomerase
 . , telomerase hTERT
 hTER hTERT
 가
 hTERT hTER telomerase subunit hTEP telomerase
 c-Myc, Mad1
 . hTEP 가
 hTEP AZT
 . c-Myc hTERT
 , hTERT
 . c-Myc hTERT
²⁷ AZT가 c-Myc c-Myc 가 hTERT
 . Mad1
 AZT 가
 hTERT , hTERT
 .
 AZT telomere 가
 telomere 가 , AZT
 telomere 가 . Telomere가
 hTERT telomerase
 . , AZT hTERT
 가 telomerase
 telomere 가
 .
 MDA-MB231 40PD
 . AZT
 telomerase 가
 . MDA-MB-231 MCF-7 ,

IC_{20} AZT , 가
 가
 . MCF-7 MDA-MB-231 telomerase
 MDA-MB-231 MCF-7 가 , telomere
 . MDA-MB-231 AZT
 telomerase 20%
 80%
 telomerase가 TRAP assay
 . AZT
 가 가
 . IC_{20}
 MCF-7 가 population doubling ,
 MDA-MB-231 telomerase telomere
 telomere가
 MCF-7 .
 AZT MCF-7
 가, 가가
 telomerase subunits telomerase
 , telomere , MDA-MB-231
 가 가

Telomerase AZT
hTERT가 , c-Myc AZT
hTER, hTEP 가
telomerase subunits telomerase telomere 가

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**The dynamics
of the telomerase activity and the telomerase subunit genes
with reverse transcriptase inhibitor treatment in human cancer cells**

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Shortening of the telomeric DNA at chromosome ends is postulated to limit the life span of human cell. In contrast, activation of telomerase, the reverse transcriptase that synthesized telomeric DNA, is proposed to be an essential step in cancer cell immortalization and cancer progression.

Several reports have described about attempts to inhibit telomerase activity using reverse transcriptase inhibitors. 3-azido-2',3'-dideoxythymidine (AZT), a reverse transcriptase inhibitor, was reported to incorporate in telomeric sequences immortalized cells in culture and shown to suppress the activity of telomerase and inhibit the cell proliferation.

In this study, we induced cancer cell senescence after long-term treatment of AZT with IC_{10} , IC_{20} dose. After inducing senescence, we investigated the dynamics of telomerase subunit (hTERT, hTER, hTEP), hTERT transcription

factors (c-Myc, Mad1), telomerase activity, and finally, telomere length in MCF-7 and MDA-MB-231 cell lines.

We demonstrated evidences of senescence, apoptosis and growth delay after AZT treatment. Also, AZT-treated cancer cells have shown inhibition of telomerase activity and shortening of telomere length in a dose- and duration-dependent way. Among telomerase subunits, hTERT and c-Myc were the first factors which changed after AZT treatment followed by the changes of hTER, Mad1 and hTEP.

In conclusion, the suppression of the hTERT and c-Myc by AZT treatment was the initial genetic phenomenon followed by the change of hTER, Mad1 and hTEP.

Key Words : telomerase, telomere, reverse transcriptase inhibitor, senescence, hTERT, hTER, hTEP, c-Myc, Mad1