

9) DNA , 2 3
¹²⁾
Radford 2.
가 Cs-137
^{13, 14)} (Mark type I, Shepherd, USA)
가 3 Gy/
¹⁴⁾
가
T- 가
¹⁵⁾ 가
가 24
³⁾
가 3. (colony formation assay, clonogenic assay)
¹⁶⁾ tetrazolium (MTT assay)
¹⁷⁾
가 , 14 100%
methanol 5 μ L 10 crystal violet 2 μ L
¹⁸⁾
가 10
가 1
50
(planting efficiency, P.E.)
(remaining fraction, S.F.)
P.E. = $\frac{\text{---}}{\text{---}} \times 100 (\%)$
S.F. = $\frac{\text{---}}{\text{---}} \times (P.E./100)$
1. 가
) PCI-1 (가 3
) PCI-13 ()
) , SNU-1066 (4.
CCRF- CEM () Annexin V-FITC Apoptosis Detection Kit (Sigma
A22 14, USA) ¹⁹⁾ Annexins
LM217 , annexin V-FITC
PCI-1, PCI-13, LM217 DMEM , SNU-
1066 CCRF-CEM RPMI-1640 가
10% fetal bovine serum (FBS) . Annexin V-FITC가
gentamycin 100 μ g 가 , 37 , 5% CO₂

2, 4, 6, 15 Gy 24, 48, 72, 96
 (PBS) 2
 1,200 rpm 5 1 × 10⁶ /mL
 1 × binding buffer 500 μL 12 × 75 mm
 propidium iodide 10 μL 가 annexin V-FITC 5 μL pro-
 10

annexin V-FITC
 propidium iodide 3 (apoptotic index, AI)
 annexin V-FITC (apoptotic fraction, AF) 가

$$A.I. = \frac{\text{annexin V-FITC}}{\text{propidium iodide}}$$

$$A.F. = \frac{A.I.}{(1 + A.I.)} \times 100 (\%)$$

5. SPSS (Release 9.01, SPSS Inc., 1999) Mann-Whitney Spearman's correlation

1. 2 Gy PCI-1, PCI-13, SNU-1066,

Table 1. Surviving Fraction at 2 Gy (SF₂) and 6 Gy (SF₆) of -Irradiation

Cell line	SF ₂	SF ₆
LM217	0.100	0.001
PCI-1	0.741	0.140
PCI-13	0.544	0.039
SNU-1066	0.313	0.019
CCRF-CEM	0.302	0.060

CCRF-CEM, LM217 0.741, 0.544, 0.313, 0.302, 0.100
 , 6 Gy 0.140, 0.039, 0.019, 0.060,
 0.001 LM217 가 PCI-1 가
 (Table 1).

2. 48 72
 가 가 (Table 2 6). PCI-1 PCI-13
 48 가 가 72 가 가
 , SNU-1066 48 가 가
 가 2 Gy 6 Gy
 . PCI-13 15 Gy 2
 Gy 6 Gy 가 가
 CCRF-CEM 24
 가 가 LM217

Table 2. Apoptotic Index according to Radiation Dose and Time after -Irradiation for LM217 Cells

Dose* (Gy)	Time after irradiation (hr)			
	24	48	72	96
0	3.58%	7.70%	10.30%	21.32%
2	16.90%	10.46%	10.42%	16.58%
4	8.60%	19.54%	16.74%	12.72%

*6 Gy was omitted because of high radiation-sensitivity of LM217

Table 3. Apoptotic Index according to Radiation Dose and Time after -Irradiation for PCI-1 Cells

Dose (Gy)	Time after irradiation (hr)			
	24	48	72	96
0	4.66%	7.86%	3.72%	7.04%
2	5.42%	7.30%	11.40%	11.84%
4	6.06%	7.44%	20.24%	20.16%
6	7.04%	11.84%	16.82%	16.36%

Table 4. Apoptotic Index according to Radiation Dose and Time after -Irradiation for PCI-13 Cells

Dose (Gy)	Time after irradiation (hr)			
	24	48	72	96
0	19.55%	11.83%	12.83%	13.90%
2	12.32%	9.61%	21.82%	21.82%
6	9.40%	7.08%	31.48%	24.26%

G2/M 가 가 .
²¹⁾ 가 .
 G2 가
 , ²²⁾ G2 III
 p53 가 가 ,
²³⁾ 가 가
²⁸⁾ Wheeler Levine
 3 8 가 ²⁹⁾
 가 가
 6 11) 가
 가 가
²¹⁾ 가 가 (가
) 가 가 ^{21, 24)} Shinomiya ,
 X 가
²⁵⁾ 가 ,
 ,
 . Algan ,
 24 가 .
 , DU-145 가
 single-hit killing 5%가
²⁶⁾ LM217
 CCRF-CEM 24
 가 SNU-1066 72 PCI-1, PCI-13,
 가 가 ,
 가 가
 가 가
 가 가
 가 ,
 가 . Levine 가
 , SF2
 SF2가
²⁷⁾ 2 Gy ,

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Abstract

Significance of Apoptotic Cell Death after γ -Irradiation

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Purpose : The objectives of this study are to investigate the significance of apoptotic death compared to total cell death after γ -ray irradiation in human H&N cancer cell lines and to find out correlation between apoptosis and radiation sensitivity.

Materials and method : Head and neck cancer cell lines (PCI-1, PCI-13, and SNU-1066), leukemia cell line (CCRF-CEM), and fibroblast cell line (LM217) as a normal control were used for this study. Cells were irradiated using Cs-137 animal experiment irradiator. Total cell death was measured by clonogenic assay. Annexin-V staining was used to detect the fraction of apoptotic death.

Results : Surviving fraction at 2 Gy (SF2) were 0.741, 0.544, 0.313, 0.302, and 0.100 for PCI-1, PCI-13, SNU-1066, CCRF-CEM, and LM217 cell lines, respectively. Apoptosis was detected in all cell lines. Apoptotic index reached peak value at 72 hours after irradiation in head and neck cancer cell lines, and that was at 24 hours in CCRF-CEM and LM217. Total cell death increased exponentially with increasing radiation dose from 0 Gy to 8 Gy, but the change was minimal in apoptotic index. Apoptotic fractions at 2 Gy were 46%, 48%, 46%, 24%, and 19% and at 6 Gy were 20%, 33%, 35%, 17%, and 20% for PCI-1, PCI-13, SNU-1066, CCRF-CEM, and LM217, respectively. The radioresistant cell lines showed more higher apoptotic fraction at 2 Gy, but there was not such correlation at 6 Gy.

Conclusion : All cell lines used in this study showed apoptosis after irradiation, but time course of apoptosis was different from that of leukemia cell line and normal fibroblast cell line. Reproductive cell death was more important mode of cell death than apoptotic death in all cell lines used in this study. But there was correlation between apoptotic fraction and radiation sensitivity at 2 Gy.

Key Words : Radiation, Apoptosis, Mitotic death