Radiobiological analysis of human lung cancer cells on the 20 MeV linac and 45 MeV cyclotron proton beam

Myung-hwan Jung[a*], Se-jin Ra[a], Jae-Keun Kil[a], Sang-Pil Yoon[a] Kye-Ryung Kim[a*]
[a] *Proton Engineering Frontier Project, Korea Atomic Energy Research Institute. Daejeon 305-353, Korea*
[*] *Corresponding author: kimkr@kaeri.re.kr*

1. Introduction

The Proton Engineering Frontier Project is making 100MeV proton linear accelerator in Korea by 2012. And it is offering now 20MeV proton beams. Proton accelerators using radiation biology research has been limited to the MC-50 cyclotron in Korea Institute of Radiological and Medical Sciences (KIRAMS) [1]. We started this work, 20MeV linear accelerator [2] used for radiation studies on biological research. In this study, we compared effect of protons both accelerated in linear accelerator and cyclotron in the tumor cells.

2. Methods and Results

2.1 20MeV Linac and 45MeV MC-50 Cyclotron.

The characteristic of two types of the proton accelerator is introduced in Table 1.

Table I: Compared of two proton accelerator

	20 MeV linac	MC-50 cyclotron
Energy	20 MeV	10 ~ 39 MeV
Beam type	Pulse	Continuous wave
Peak current	1 ~ 20 mA	-
Average current	50 nA ~ 1 μA	~ 10 nA
Repitition rate	0.1 ~ 60 Hz	-

2.2 Cell Culture

Human lung adenocarcinoma cells (A549) was maintained in Dulbecco's Modified Eagles Medium (DMEM, Hyclone, Logan) supplemented with 10% FBS (Hyclone, Logan) and 1% antibiotics.

2.2 Proton Beam Irradiation

Cells were irradiated with 14 MeV proton beams from the MC-50 cyclotron and 20MeV linac. For the same irradiation condition, we use energy degrader adjusted 14 MeV in MC-50 cyclotron. Anchored cells were irradiated in a T-12.5 flask or 96-well plate filled with media, and placed on a beam stage. Flasks were oriented such that the growth surface was orthogonal to the horizontal beam entering of the flask [3].

.

2.3 Cell Viability Assay

A549 cells were plated at $5x10^4$ cells per well in DMEM media supplemented with 10% FBS and 1X antibiotics for 24h, then irradiated with proton beams at 5 to 70 Gy doses and further cultured for 5 day. Cell viability was determined using 3-4,5-dimethylthiazon-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay at 540 nm with a micro plate reader. And irradiated A549 cells were plated at $1x10^3$ cells per 100mm culture dish. 9 days after, counted colony number.

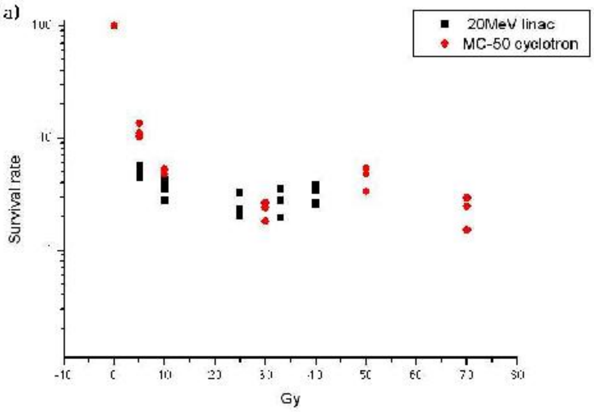
2.3 Statistical Analysis

ANOVA analysis was performed for statistical significance. P values less than 0.05 were considered significant.

2.4 Comparison of Cell Death Effect in Two Type Proton Accelerators

When A549 cells were irradiated with proton 5 to 70 Gy, colony formation assay (Fig. 1) result shows that cell viabilities were inhibited by 97-90% of control cells in 9 day after irradiation.

(a)



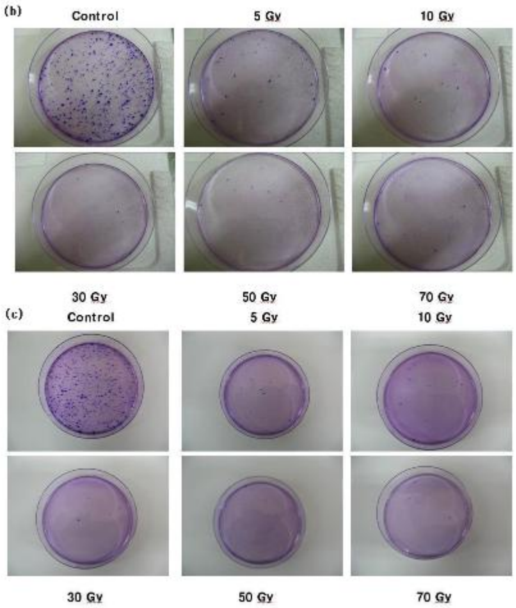


Fig. 1. Effects of proton beam on A549 lung carcinomas. (a) Effects of cell death by 20MeV linac is very similar to a MC-50 cyclotron. (b) The pictures of colony formation by 20 MeV linac proton beam irradiation. (c) The pictures of colony formation by MC-50 cyclotron proton beam irradiation.

We confirmed effect of A549 cell death by MTT assay. MTT data shows cell viabilities were inhibited by 20-10% and 70-60% of control cells in dose dependent manner 24h and 72h after irradiation. (Fig. 2)

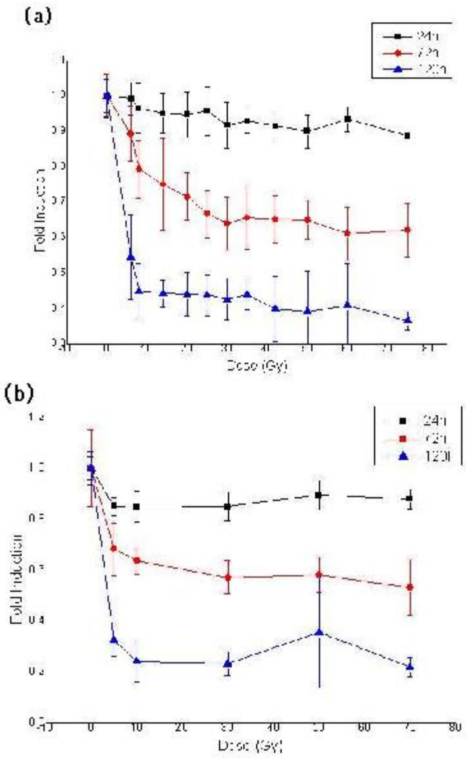


Fig. 2. Proton beam inhibited cell survival rate on A549 cells. (a) Survival rate of A549 cells by 20 MeV linac beam irradiation. (b) survival rate of A549 cells by MC-50 cyclotron.

3. Conclusion and discussion

In this work, the first approaches to radiobiology with protons including pulse beam and continuous wave system compared at biological effects. We experimented adjusting the dose rate and energy. In the case of 20MeV linac, dose rate is higher than cyclotron because of pulse length is short. But, the results show two proton beams are very similar effect of cell killing ability in 5 to 70 Gy dose range. However, we are going to experiment in a low dose level fewer than 5 Gy because 5 to 70 Gy is high dose level.

REFERENCES

[1] Kye-Ryung. Kim, Yong-Sub Cho, In-Seok Hong, bum-Sik Park, Sang-Pil Yun, Han-Sung Kim, Hong-Joo Kim and Jung-Ho So. Proton beam energy measurement using semiconductor detectors at the 45 MeV test beam line of PEFP, Proc. Of the PAC07, pp 4126-4128, 2007

[2] Yong-Sub Cho, Hyeok-Joong Kwon, Ji-Ho Jang, Han-sung Kim, Kyung-Tae Seol, Dae-Il Kim, Young-Gi Song, In-Seok Hong. The PEFP 20-MeV proton linear accelerator. Journal of the Korean Physical Society. 52: 721-726, 2008

[3] Kheun-Byeol Lee, Jong-Soo Lee, Jeen-Woo Park, Tea-Lin Huh and You Mie Lee. Low energy proton beam induces tumor ell apoptosis through reactive oxygen species and activation of caspases. Experimental and Molecular Medicine, 40: 118-129, 2008