Preliminary study on the mouse tumor growth suppression by proton beam irradiation

M. H. Jung [a], S. K. Lee [a], H. W. Kim [a], T.K. Yang [b], Y. M. Lee [c], K.R. Kim [a*]
[a] *Proton Engineering Frontier Project, Korea Atomic Energy Research Institute, Daejeon, 305-353 Korea.*
[b] *Korea Institute of Radiological and Medical Sciences, Seoul, 139-706 Korea.*
[c] *School of Life Sciences and Biotechnology, Department of Natural Sciences, Kyungpook National University, Daegu, 702-701 Korea*
[*] *Corresponding author: kimkr@kaeri.re.kr*

1. Introduction

The proton beam is known as a one of the powerful tools for the treatment of tumor tissues. Interest on the proton therapy has been increased in past few years. [1, 2] Compared to conventional radiation therapy methods such as using X-ray or Gamma-ray, proton beam have less side effects after incidence to the body because of its specific energy delivery system [3, 4]. It is the reason why proton therapy has become more widely used for tumor treatment for patients suffering from various kinds of tumors. As far as in-vitro experiment it concerning tumor cell death by proton beam irradiation, it has been mainly performed because the in-vivo experiment system was not established [5]. Requirements for domestic researchers who want to use proton beam for the in-vivo study are increased. In order to meet these demands, we have established an in-vivo experiment system. In this paper, we used the Lewis Lung Carcinoma (LLC) mouse model to determine in-vivo tumor size decreases by proton beam. And we confirmed the possibility of the developed system for the application to use in-vivo experiments in the field of biomedical sciences.

2. Methods and Results

2.1 Cell culture and animal maintenance

Lewis lung carcinoma cells (LLC) was maintained in Dulbecco's Modified Eagles Medium (DMEM, Hyclone, Logan) supplemented with 10% FBS (Hyclone, Logan) and 1% antibiotics. Animal maintenances were approved by the Institutional Administrative Panel on Laboratory Animal Care. Tumorigenesis was performed on C57BL/6J mice over 6 weeks.

2.2 Tumorigenesis experiment in mouse

$5x10^5$ of LLC cell were mixed with Matrigel in final volume 200 μl (ratio 1:1) and immediately inoculated subcutaneously at the right flank of C57BL/6J. Tumor growth were measured with caliper every 1 days using a formula, volume (V)=height x length x depth (cm^3)

2.3 MC-50 cyclotron and in-vivo experimental devices.

The 45 MeV proton beam was produced from Low Energy Proton Therapy (LEPT) line of the MC-50 Cyclotron (Scanditronix, Sweden) at the Korea Institute of Radiological and Medical Sciences (Seoul, Korea). Mice were fixed by mouse holder which was placed at the end of the beam line. The in-vivo experiment system consists of a SOBP (Spread out Bragg Peak) system, a ridge-filter type modulator, a range shifter, a collimator, a bolus, a mouse holder and a depth-does measurement system (figure 1), etc.

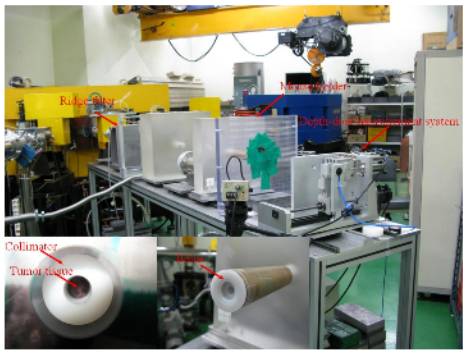

Figure 1 LEPT beam line and in-vivo experiment system.

2.4 Proton beam irradiation and dosimetry

8 days after inoculation, the mice were irradiated with a proton beam by using a LEPT prototype system installed at the MC50 Cyclotron. We aligned the center of the tumor mass to the beam line center using the mouse holder. And the bolus was used for control of the penetration depth according to the shape of the tumor mass. Mice were irradiated at single dose level of 20 and 40 Gy. The average dose rate was 0.13 Gy/s. Using the Depth-does Measurement System confirmed Bragg peak and SOBP (Figure 2).

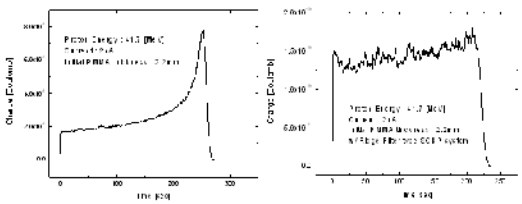

Figure 2 Depth-dose Measurement System confirmed Bragg peak (left panel) and SOBP (right panel).

2.5 Inhibition of tumor growth in lung carcinoma in C57BL/6J mice by proton beam irradiation

After the LLC were inoculated in the flank of the mice subcutaneously, proton beam irradiated. Control mice showed rapid increase of tumor growth. However, the proton beam inhibited tumor growth of lung carcinomas when irradiated with doses of 20 and 40 Gy (Figure 3). Moreover, proton beam irradiation groups were increased the survival rate compared to control mice (Figure 4).

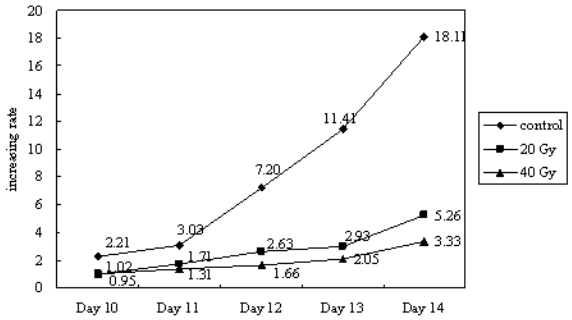

Figure 3 Proton beam attenuated tumor growth in mice models. Each mouse exposed proton beam with 20 Gy or 40 Gy. Tumor growth were measured with caliper from Day 10 to Day 14 using the formula V=height x length x depth (mm^3).

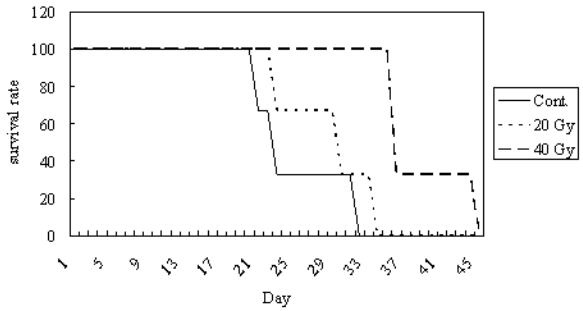

Figure 4 Proton beam contributed to survival in mice tumor models. Fig. 2 shows a marked advantage in terms of survival time overall, i.e., 45 days vs. 34 days.

3. Conclusions

Proton beam radiation has successfully inhibited tumor growth and increased survival rates using a developed system. However it was very difficult to make a precise treatment planning because the growth rate of the mouse tumor was too fast. To solve this problem, next we will apply it to a human cancer using nude mouse. And the effort is necessary to improve accuracy of planning and precision of treatment.

REFERENCES

[1] Levin WP, Kooy H, Loeffler JS, DeLaney TF. Proton beam therapy. Br J Cancer 2005;93:849-54
[2] MacDonald SM, DeLaney TF, Loeffler JS. Proton beam radiation therapy. Cancer Invest 2006;24:199-208
[3] Belli M, Bettega D, Calzolari P, Cera F, Cherubini R, Dalla Vecchia M, Durante M, Favaretto S, Gialanella G, Grossi G, Marchesini R, Moschini G, Piazzola A, Poli G, Pugliese M, Sapora O, Scampoli P, Simone G, Sorrentino E, Tabocchini MA, Tallone L, Tiveron P. Inactivation of human normal and tumour cells irradiated with low energy protons. Int J Radiat Biol 2000;76:831-9
[4] Bettega D, Calzolari P, Chauvel P, Courdi A, Herault J, Iborra N, Marchesini R, Massariello P, Poli GL, Tallone L. Radiobiological studies on the 65 MeV therapeutic proton beam at Nice using human tumour cells. Int J Radiat Biol 2000;76:1297-303.
[5] Lee KB, Lee JS, Park JW, Huh TL, Lee YM. Low energy proton beam induces tumor cell apoptosis through reactive oxygen species and activation of caspases. Exp Mol Med. 2008; 29:118-29.