The Preliminary Study of an In-vivo Proton Therapy and a Cancer Diagnosis Using Optical Coherence Tomography

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1. Introduction

The Korea Electronics and Telecommunications Research Institute (ETRI) have been developing a 100MeV high power laser-derived compact proton therapy machine. The 100MeV proton therapy machine has been applied to the eye cancer because it is not enough to treat deep seat cancer. The eye cancer diagnosis research using the OCT in parallel with development of the 100MeV proton therapy machine has been performed. If the technology of cancer diagnosis using OCT can be developed, the OCT could be an alternative to CT or MRI because OCT has a high resolution than CT or MRI [1]. It is very attractive that cancer diagnosis using OCT can reduce unnecessary radiation to the cancer patients.

In this research, we studied the tumor cell apoptosis and the feasibility of the tumor diagnostics using an OCT before applying to the treatment of eye cancer when developed high power laser-derived compact proton therapy machine.

2. Methods and Results

2.1 Introduce LHβ Tag mice from MMRRC and build up the breeding facility

LHbeta-Tag mice were imported from MMRRC (Mutant Mouse Regional Resource Center, USA) and In this model, tumor appears at 4 weeks of age and fills the available orvit volume by 16 weeks of age [2]. We installed the *LHbeta-Tag* mice breeding facility added on the existing facilities of the PEFP (proton Engineering Frontier Project). To maintain a stable mice strain and to prevent sudden deaths of mice caused by the malfunction of the thermostatic system we installed a remote notification system.

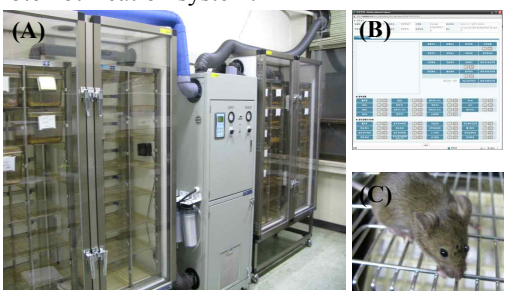

Fig. 1. (A) Breeding facility. (B) Remote notification system. (C) *LHbeta-Tag* mouse.

2.2 Cell Culture

Y-79(human retinoblastoma cell), MOLT-4(human leukemia cell), U-937(histocytic lymphoma cell) and SNU-16(human gastric cancer cell) were maintained in RPMI 1640 medium (Hyclone, Logan) supplemented with 10% FBS (Hyclone, Logan) and 1x antibiotics.

2.3 Proton Beam Irradiation

Tumor cells were irradiated with 45MeV proton beams from the MC-50 cyclotron at the KIRAMS (Korea Institute of Radiological & Medical Sciences). Anchored cells were irradiated in an E-tube or 0.2ml tube filled with media, and placed on a beam stage [3].

2.4 Cell Viability Assay

1 to 10 Gray doses proton irradiated cells were plated at $5x10^4$ cells per well in RPMI media and cultured for 5 days. Cell viability was determined using CCK-8 (Cell Counting Kit-8, Dojindo) and soft agar colony assay. Figure 2 and 3 shows that cancer cell death is occurred by proton beam irradiation and different of radio-sensitivities depending on the tumor cell origin were observed. The histiocytic lymphoma has the most sensitivity and gastric cancer cell has the lowest. Approximately 70% of cells were occurred apoptosis in the 10 Gy dose 120h after proton beam irradiation.

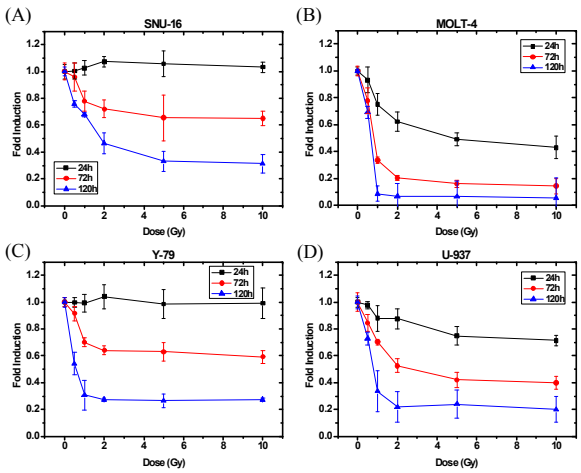

Fig. 2. CCK-8 data. (A) SNU-16. (B) MOLT-4. (C) Y-79. (D) U-937

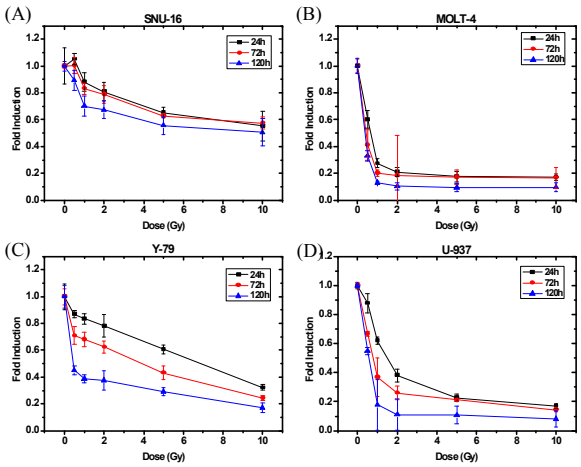

Fig. 3. Soft agar colony formation assay data. (A) SNU-16. (B) MOLT-4. (C) Y-79. (D) U-937

2.5 In-Vivo Experiment and Results

We performed in-vivo experiment using nude mouse S.C. tumor models. 11 days after inoculation of tumor cells, mice were irradiated by proton beam with 20 Gray doses. Rapid increase of tumor size in the control mice was shown, on the other hand, proton inhibited tumor growth in the proton beam irradiated group was observed. We confirmed this result by taking PET/CT images (figure 4).

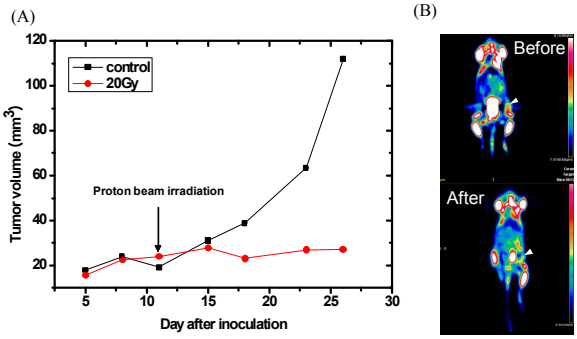

Fig. 4. Proton beam attenuated tumor growth in nude mice. (A) Tumor growth curve. (B) CT data.

2.6 The Possibility of Tumor diagnosis using OCT

To check the possibility of OCT cancer diagnosis, we made the tumor in ear of mice. 3 days after inoculation, we obtained OCT images and identify the difference of tumor tissue and normal tissue. We compared the OCT image with the histological image at the same position where the cancer was located (figure 5).

The biggest challenge of OCT retinal imaging of the mouse eye comes from the small size of the eye and the very small pupil. The small pupil size of the mouse eye makes the alignment for light delivery to the eye formidable [4]. It also limits the amount of light reflected from the retina and thus decreases the signal—to-noise ratio (SNR). So we made prototype device to fix the mouse and OCT. The relative position of mouse to OCT probe can be controlled for six-axis manually by using developed positioning system (Figure 6).

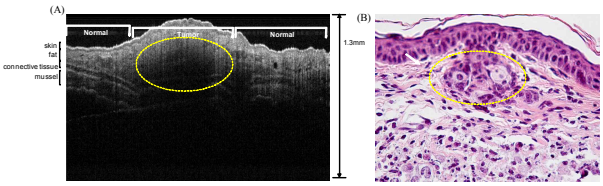

Fig. 5. (A) OCT image. (B) histological image

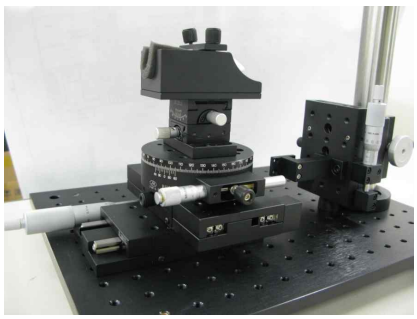

Fig. 6. Prototype of mouse fixation device

3. Conclusions

Through this research, *LHbeta-Tag* mice breeding, radiation sensitivity measurement of the retinoblastoma, in-vivo experiments of tumor tissue and OCT image acquisition were performed. These results will be useful for the development of high power laser-derived compact proton therapy machine and for the application of OCT diagnostic devices to the eye tumor treatment.

4. Acknowledgement

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REFERENCES

[1] R.A. Leitgeb. Optical coherence tomography-high resolution imaging of structure and function, Conf. Proc. IEEE Eng. Med. Biol. Soc. 2007;530-532 (2007)

[2] J.J. Windle, D.M. Albert and J.M. O'Brien. Retinoblastoma in transgenic mice. Nature 343;665-669 (1990)

[3] K.B. Lee, J.S. Lee, J.W. Park, T.L. Huh and Y.M. Lee, Low energy proton beam induces tumor cell apoptosis through reactive oxygen species and activation of caspases, Exp. Mol. Med. 40;118-129 (2008)

[4] Ruggeri M., Wehbe H., Jiao S., *et al*., In vivo three-dimensional high-resolution imaging of rodent retina with spectral-domain optical coherence tomography. Invest. Ophthalmol. Vis. Sci. 48(4):1808-14 (2007)