Construction of radiation – induced metastasis model *in vivo*

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1. Introduction

In treatment of cancer, distant metastases are important limiting factor because an estimated 50% of all cancer patients will develop metastases, and the metastases are major causing of cancer treatment failure [1]. Recently a few reports indicated γ−radiation induced an increase of invasiveness of several cancer cells. In this study, we had tried to show the possibility that radiation could also induce metastasis *in vivo* system. To prove our hypothesis, we constructed primary tumor by using C6-TL transfectant cell line expressing HSV1-tk and firefly luciferase (fLuc), and then γ-radiation was treated to xenografts locally. Treatment of γ-radiation to primary C6-TL xenografts of mice reduced size of xenografts and elongated survival of mice than those of mock control mice. But we also show that γ-radiation treatment was followed by the growth of dormant metastases in various organs including lung and intestine after 2-4 weeks of γ-radiation treatment. When bioluminescence imaging indicated growth of tumor in organs in mice, we sacrificed the mice and repeat acquired bioluminescence imaging after repeatedly. These images presented tumor growth locations exactly in organs. Because metastatic tumor candidates have morphology of foci, biopsies were performed for histological analysis or PCR analysis to confirm metastases. In most foci, histological analysis indicated several features of typical cancer tissue and PCR analysis showed present of fLuc gene in metastases. Detection of fLuc gene in metastases indicated these foci were originated from primary C6-TL xenografts, and the results suggest that γ-radiation could promote metastasis *in vivo* as well as *in vitro* system. Although we need to understand changes of intracellular signaling or physiological phenomena of the radiation-induced metastasis yet, these results also imply that γ-radiation treatment only to cancer patients need to pay attention carefully, and development of new drug to block radiation-induced metastasis is required.

2. Methods and Results

2.1. Formation of Xenograft and Radiation therapy

1 X 10^6 of C6-TL cells were injected subcutaneously into 6-weekold BALB/cAnNCrj-nu/nu strain mice (Charles RiverJapan, Inc., Tokyo, Japan) to construct xenograft as described in previous report of Park *et al*[2]. When these xenografts reached more than 100 mm^3, radiation was treated to xenograft with 10 Gy at a day during 5 days but not treated to mock control group.

2.2 Detection of Tumor size and Survival of Mice

Tumor sizes and survival curves of control or radiation-treated group were detected over 62 days.

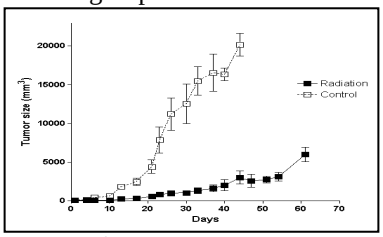


Fig. 1. Detection of Tumor Size. Radiation (black square) indicates γ-radiation-treated group (n=5), and control (white square) represents no radiation- treated group (n=4).

Tumor sizes of each mouse were detected as long and short axis length, and calculated as followed formula
: (short axis length^2 x long axis length)/2

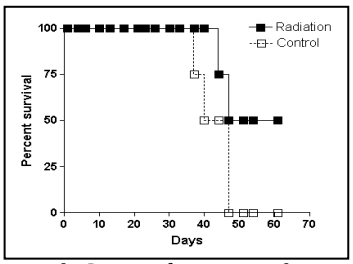
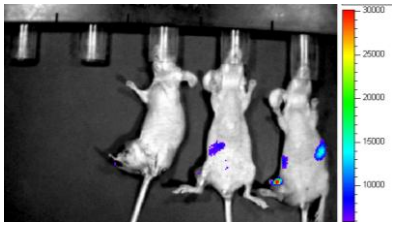


Fig. 2. Survival Curve of mice. Radiation (black square) indicates γ-radiation-treated group (n=5), and control (white square) represents no radiation- treated group (n=4)

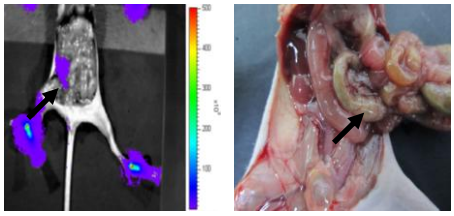
2.3 Bioluminescence imaging acquisition

Bioluminescence imaging was performed with CCD camera mounted in a light-tight specimen chamber (IVIS200, Xenogen, CA, USA). For in vivo imaging, mice were given i.p to 100 μL of 2.5 mg/100 μL D-luciferin potassium salt, and anesthetized with 2% isoflurane. Imaging and quantification of signals were controlled by the acquisition and analysis software Living Image V. 2.50 (Xenogen) as as described in previous report of Jang *et al* [3].

(A)



B)



(C)

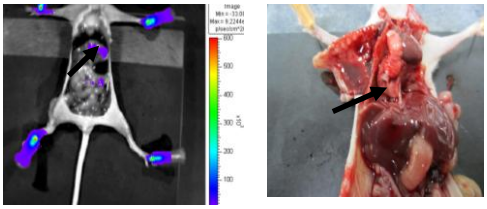
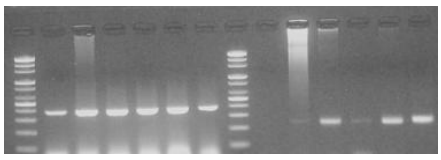


Fig. 3. Bioluminescence imaging. These images were detected after 3 weeks of radiation treatment. (A) Left: Control, Middle and right mice: 10 Gy –irradiated mice. (B) Metastatic foci in intestine (black arrow) (C) Metastatic foci in lung (black arrow)

2.4.PCR analysis

Total RNA was isolated from metastatic foci, and then the total RNA was used as a template to produce cDNA using the SuperScript III First-Strand Synthesis for RT-PCR (Invitrogen, CA, USA). The synthesized cDNA was amplified using Taq DNA polymerase (iNtRON) with the following primers: forward primer for fLuc was 5'-CGC CTT GAT TGA CAA GGA TGG and reverse primer was 5'- GGC CTT TAT GAG GAT CTC TCT. Forward primer for β-actin was 5'-GTG GGG CGC CCC AGG CAC CAG GGC and reverse primer was 5'-CTC CTT AAT GTC ACG CAC GAT TTC.



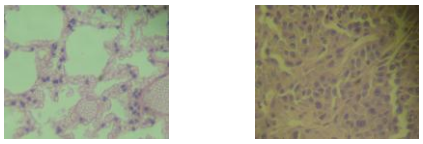
1: negative
2: negative
3: intestine
4: intestine
5: lung
6: lung

Fig. 3. RT-PCR of metastatic foci. 1 and 2 lanes: negative sample, 3 and 4 lanes: metastatic foci from intestine, lane 5 and 6: metastatic foci in lung.

2.5.Histological analysis

To perform histological analysis, metastatic foci were fixed with formaldehyde and embedded in paraffin block. The tissues were stained H and E staining solution.

(A)



(B)

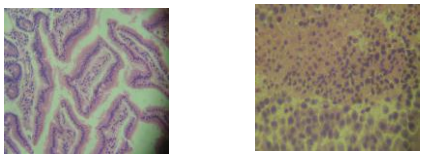


Fig. 4. Histological analysis of metastatic foci. (A) lung. Left: tissue of normal lung, Right: metastatic foci in lung (B) intestine. Left: tissue of normal intestine, Right: metastatic foci in intestine.

3. Conclusions

C6-TL transfectant cell line expressing HSV1-tk and firefly luciferase (fLuc) could construct xenograft in mice, and radiation treatment also could reduce sizes of each xenograft. But radiation treatment only could not eliminate xenograft perfectly and induce metastasis to multi-organ including lung and intestine. Metastatic foci were observed with bioluminescence imaging, and confirmed with RT-PCR for detection of fLuc gene and histological analysis. These results also suggest that radiation could induce metastasis *in vivo* system and these phenomena could be used as *in vivo* model for development of anti-metastasis drug blocking radiation-induce metastasis.

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