Radiation promotes cancer cell metastasis via EMT induction in mouse model.

[a]Jong Kuk Park, [b]Su Jin Jang, [a]Sung Wook Kang, [c]Sunhoo Park, [a]Sang-Gu Hwang, [d]Wun-Jae Kim, [b]Joo Hyun Kang, and [a]Hong-Duck Um[†].
[a]*Department of Radiation Cancer,* [b]*Molecular Imaging Research Center,* [c]*Laboratory of Radiation Pathology, Korea Institute of Radiological and Medical Sciences 215-4, Gongneung-Dong, Nowon-Gu, Seoul, 139-706, Republic of Korea,* [d]*Department of Urology, College of Medicine, Chungbuk National University, Chungbuk, Korea*
[†]Corresponding author: hdum@kcch.re.kr

1. Introduction

In the present study, we attempted to establish whether γ-IR-induced invasion and metastasis are stimulated in our *in vitro* C6L cell line and *in vivo* systems, and further identify the associated changes in signal pathways or mice physiology. We constructed an animal model system with a view to clarifying the intracellular molecular events underlying the promotion of metastasis after γ-IR treatment for primary cancer and developing effective anti-metastatic reagents. Our results demonstrate that γ-IR treatment of cancer cell lines and mice xenografts triggers invasion and metastasis. In particular, γ-IR-treated cancer cells or mouse xenografts and metastatic lesions in mice bearing γ-IR-treated xenografts also display typical EMT marker expression patterns, such as increased vimentin or MMP-2 expression, decreased E-cadherin, and enhanced activity of MMP-2. Our results collectively suggest that γ-IR-induced invasion or metastasis results from induction of EMT, and inhibition of EMT may thus be a means to enhance the effectiveness of radiation therapy.

2. Methods and Results

2.1. Γ-IR promotes invasiveness via induction of EMT in vitro and in vivo.

In this study, we investigated the possibility that γ-IR promotes metastasis in an in vivo murine model system. We constructed a firefly luciferase (fLuc)-expressing C6L transfectant cell line derived from the rat glioma cell line, C6. Construction of the C6L cell line was confirmed with PCR analysis using firefly luciferase primer and GAPDH (Fig. 1A). Enhanced invasion of C6L cells following γ-IR treatment was detected in a dose-dependent manner based on quantitative analysis using matrigel-coated transwells (Fig.. 1B). Treatment with 3 Gy of γ-IR induced the maximum increase in C6L cell invasion by more than two-fold. But treatments of γ-IR in a dose-dependent manner did not affect C6L cell survival (Fig. 1C). In another preliminary experiment, we treated xenografts with 10 Gy of γ-IR, and isolated the tissue for IHC analyses with EMT markers (Fig.1D).

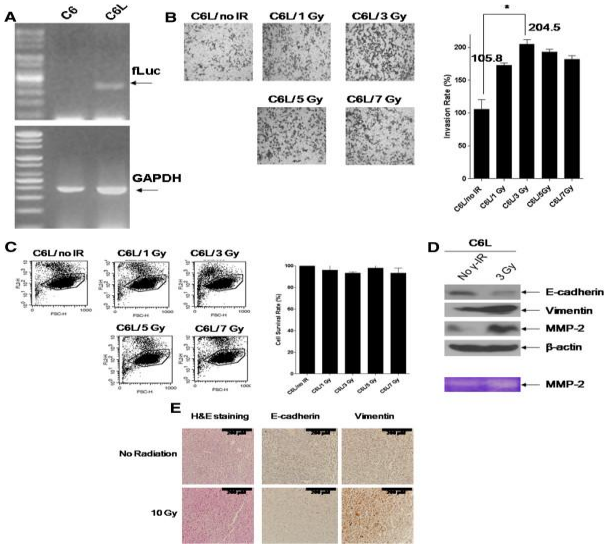

Fig. 1. Γ-IR promotes invasion of C6L cells through induction of EMT.

2.2. Γ-IR caused regression of tumors and prolonged survival but did not block death of the in vivo model perfectly.

To further establish whether γ-IR induces metastasis of cancer *in vivo*, we constructed xenografts with 5 X 10^5 C6L cells irradiated with γ-IR, as described in Materials and Methods. (Fig. 2A and 2B). We calculated the time-periods needed for xenograft sizes of each group to reach 2500 mm^3 (Fig. 2B). Notably, the control group reached this size in 4 days and the γ-IR treatment group in 45.7 days, revealing a 41.3-day growth delay in terms of xenograft size (Fig. 2B). With regard to survival rates, 50% of γ-IR-treated mice survived until day 61, while control mice did not survive for more than 44 days (Fig. 2C)

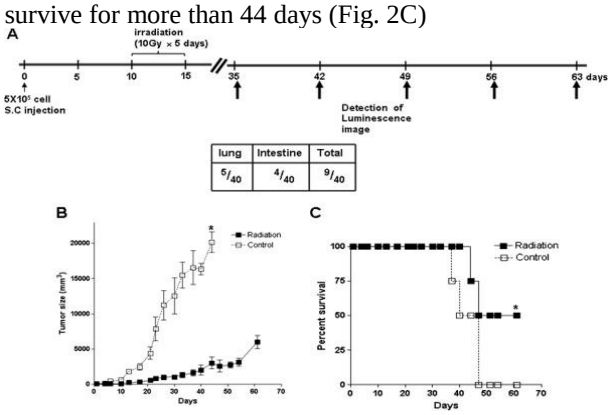

Fig. 2. Treatment with γ-IR affects tumor size and percentage survival in mice.

2.3. Metastases following γ-IR treatment were detected in the murine in vivo model.

We constructed xenografts of 40 mice in total and treated them with γ-IR, as shown in Fig. 2A. Bioluminescence images were detected, as described in Materials and Methods. Bioluminescence signals in the body trunks of mice, except the xenograft sites, were observed in 9 (22.5%) among the 40 mice. *Ex vivo* bioluminescence images were detected in 3 among 5 mice displaying signals in the chest region (Fig. 3A). Lung metastases were observed in *ex vivo* bioluminescence images, but brain metastases were not. Relationship analysis of fLuc activity with tumor volume *in vivo* indicated a time-dependent increase in signal intensity (Fig. 3C and 3D).

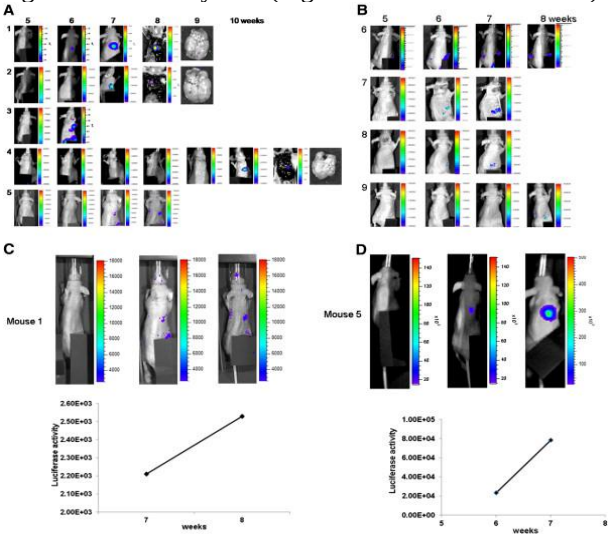

Fig. 3. Detection of metastasis of γ-IR-treated mice using bioluminescence imaging.

2.4. Metastases in the animal model may be accompanied by EMT induction

Although *ex vivo* bioluminescence image analysis failed to completely reveal bioluminescence signals in mice subjected to whole autopsy, several lesions estimated as neoplasms in the lung and intestine were observed with visual assessment (Fig. 4A). Lesions were further analyzed by PCR with *fLuc* or *GAPDH* primers (Fig. 4B) and histological experiments (Fig. 4C). As shown in Figure. 4B, each lesion expressed the fLuc gene, indicative of its origin from C6L xenografts as a primary cancer. H & E staining in histological analysis revealed the formation of solid tumors in normal lung and intestine tissues (Fig. 4C). These results suggest that γ-IR promotes metastasis *in vivo* as well as *in vitro*, and induction of cancer spread by irradiation is accomplished randomly. To identify changes in intracellular signaling or physiological phenomena occurring in γ-IR-induced metastasis, we assessed the expression of ECM markers in histological tissue samples (Fig. 4D).

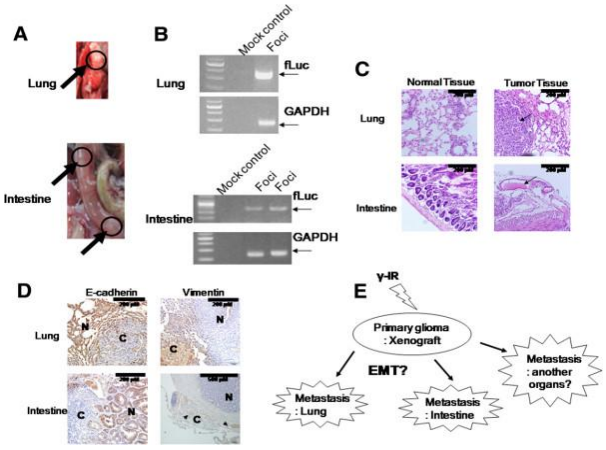

Fig. 4. Histological or genetic analysis of metastatic lesions.

3. Conclusions

This study is focused on the construction of an animal model for the development of inhibitor to block the metastatic process which occurs during radiotherapy. Γ-IR treatment did not block the occurrence of metastases in mice containing xenografts of C6L cells. Induction of EMT markers was detected in γ-IR – treated cells, xenografts, and metastatic lesions in mice. Therefore, our results also suggested EMT might be one of the major therapeutic targets to block metastasis

REFERENCES

[1] Gupta GP, Massagué J: Cancer metastasis: building a framework. *Cell* 2006, 127(4):679-695.

[2] Weber GF: Molecular mechanisms of metastasis. *Cancer Lett* 2008, 270(2):181-190.

[3] Friedl P, Wolf K: Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer* 2003, 3(5):362-374.

[4] Pavelic SK, Sedic M, Bosnjak H, Spaventi S, Pavelic K: Metastasis: new perspectives on an old problem. *Mol Cancer* 2011, 10:22.

[5] Box C, Rogers SJ, Mendiola M, Eccles SA: Tumour-microenvironmental interactions: paths to progression and targets for treatment. *Semin Cancer Biol* 2010, 20(3):128-138.

[6] Pani G, Galeotti T, Chiarugi P: Metastasis: cancer cell's escape from oxidative stress. *Cancer Metastasis Rev* 2010, 29(2):351-378.

[7] Gatenby RA, Gillies RJ: Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 2004, 4(11):891-899.