Induction of premature senescence by single and fractionated irradiation in human cancer cell line and xenografted mice model

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1. Introduction

Radiation therapy (RT) is one of the best therapeutic strategies for cancer treatment. The cellular responses to ionizing radiation (IR) are varied ranging from cellular senescence to apoptotic cell death [1]. To increase the efficacy of IR treatment is a major issue of radiation biology. From the point of view, the induction of premature senescence using the therapeutic dose of IR could be a promising treatment for tumors [2]. The aim of this study is whether the premature senescence could contribute to cancer treatment by irradiation.

2. Methods and Results

2.1 IR induces premature senescence in cancer cell lines

To investigate IR-induced premature senescence in cancer cell lines, we examined colony forming ability, senescence-specific morphologies and senescence-associated β galactosidase (SA-β-Gal) activity in human lung cancer cells H460 exposed to single irradiation (SR) of various IR doses or fractionated irradiation (FR) of the therapeutic dose (2Gy). Colony forming ability was significantly decreased in IR-exposed H460 cells. The SA-β-Gal activity, specific marker for cellular senescence was also progressively increased accompanying large and flat morphological changes with time in a dose-dependent manner.

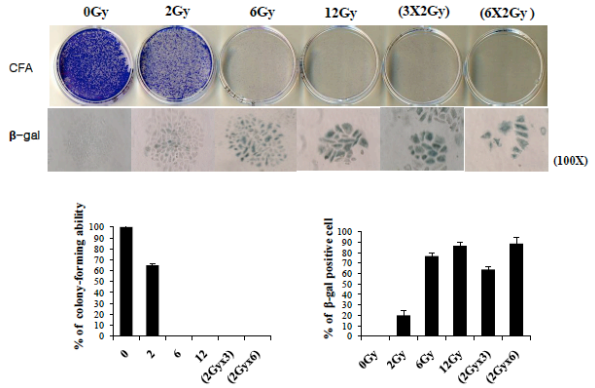

Fig. 1. Determination of premature senescence in SR- or FR-exposed H460 cells using colony forming assay and SA-β-Gal positivity.

Western blot analysis revealed molecular changes related with cell cycle arrest such as the decrease of phospho-pRb, the accumulation of p53 and the increase of p21 in IR-exposed cells. Together with these changes, we also tested CD, eEF1A1, eEF1B2, DEC1 and DcR2, previously reported as senescence markers [4-5] (Fig. 2).

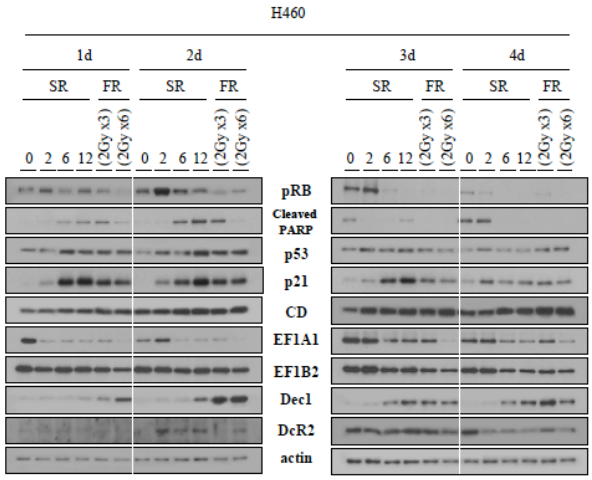

Fig. 2. Western blot analysis was performed with specific antibody of each protein in cell lysates which were obtained at 1 to 4 days post-irradiation.

2.2 IR-induced premature senescence inhibits tumor growth of xenografted mice

We developed a mouse xenograft model of human lung cancer by subcutaneously transplanting H460 lung cancer cells in athymic nude mice. When xenograft tumor volume was reached to 200 ~ 250 mm^3, xenograft regions of mice were exposed to SR of IR (2, 6, or 12 Gy) or FR (3 x 2 Gy or 6 x 2 Gy) (Fig. 3).

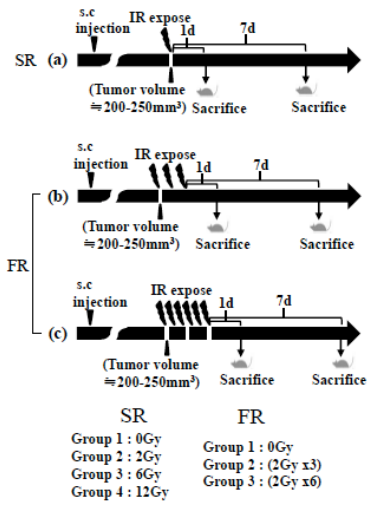

Fig. 3. Schematic drawing of *in vivo* experimental scheme

The volume of xenograft tumor was measured and compared between different groups everyday up to 7 days (Fig. 4). IR exposure retarded xenograft tumor growth drastically depending on radiation dose. Similarly to cancer cell lines, when single radiation (6 Gy or 12 Gy) was fractionated with 2 Gy three times or six times, xenograft tumor growth was inhibited with same efficiency, showing the correlation between *in vitro* and *in vivo*.

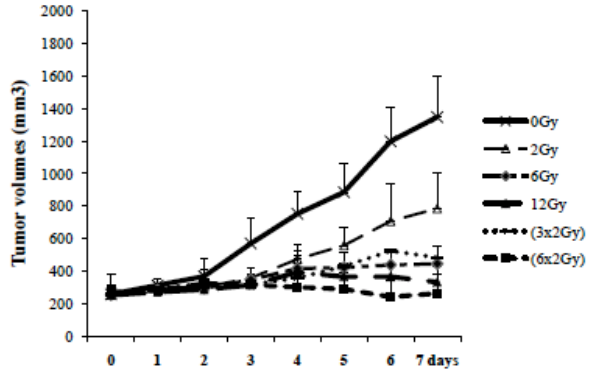

Fig. 4. Tumor volume was assessed in lung cancer cell-xenografted mice after IR exposure. When xenografts reached a volume of 200-250 mm^3, mice were exposed to IR and tumor volumes were measured every day up to 7 days.

2.3 Validation of cellular proliferation and premature senescence in vivo

To determine the efficacy of IR to regression of xenograft tumor, we validated cellular proliferation using immunohistochemistry of Ki-67 and premature senescence using SA-β-Gal staining, respectively. Cellular proliferation was decreased either SR- or FR-exposed tissue in dose- and time-dependent manners. Positivity of SA-β-Gal stain was detected evidently in tissues which were exposed to either SR or FR at 7 day after irradiation in dose-dependent manner.

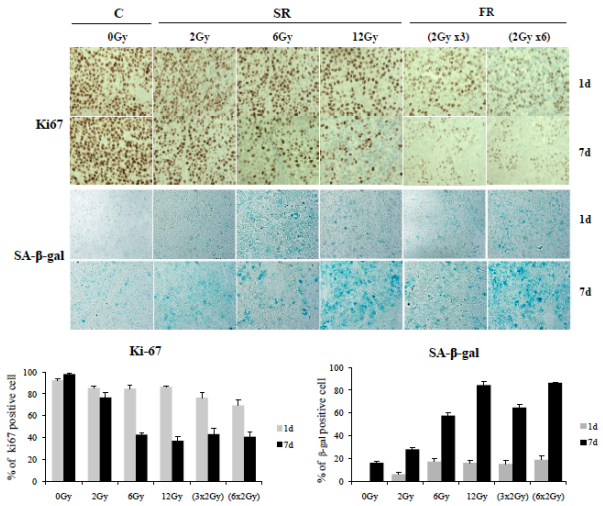

Fig. 5. Immunohistochemical analysis of Ki-67 and SA-β-Gal staining in tumor-section derived from xenografted mice which were exposed to either SR or FR, and quantitative analysis (*lower graphs*)

These results indicate that IR-induced inhibition of cell proliferation may due to induction of premature senescence and FR (3 x 2 Gy or 6 x 2 Gy) could efficiently induce premature senescence as much as SR (6 Gy or 12 Gy) of high dose.

3. Conclusions

In this study, we attempted to find out whether IR treatment can induce premature senescence both in cultured cancer cell lines and xenografted mice model. Moreover, we compared the efficiency of IR treatment between single dose and fractionated dose. We found that IR treatment could induce more senescence rather than apoptosis both *in vitro*, cancer cell line H460 and *in vivo*, tumor tissues generated in xenografted mice. Both single and fractionated radiation decreased tumor size of xenografted mice with similar efficiency when their total dose was same. Taken together, these data would be useful to make a clinical strategy to treat cancer using IR

REFERENCES

[1] K. Chu, N. Teele, M.W. Dewey, N. Albright, and W. C, Dewey, (2004). Computerized video time lapse study of cell cycle delay and arrest, mitotic catastrophe, apoptosis and clonogenic survival in irradiated 14-3-3sigma and CDKN1A (p21) knockout cell lines. Radiation Research, Vol 162, pp.270-286, 2004.

[2] D.A. Gewirtz, S.E. Holt, and L.W. Elmore, Accelerated senescence: an emerging role in tumor cell response to chemotherapy and radiation. Biochemical Pharmacology, Vol. 76, pp947-957, 2008.

[3] G.P. Dimri, X. Lee, G. Basile, M. Acosta, G. Scott, C. Roskelley, A biomarker that identifies senescent human cells in culture and in aging skin in vivo, The National Academy of Sciences of the USA,Vol. 92, pp.9363-9367, 1995.

[4] H.O. Byun, N.K. Han, H.J Lee, K.B. Kim, Y.G. Ko, G. Yoon, Y.S. Lee, S.I. Hong, and J. S. Lee, Cathepsin D and eukaryotic translation elongation factor 1 as promising markers of cellular senescence. Cancer Research Vo. 69, pp4638-4647, 2009

[5] M, Collado, J. Gil, A. Efeyan, C. Guerra, A.J. Schuhmacher, M. Barradas, A. Benguria, A. Zaballos, J.M. Flores, M. Barbacid, D. Beach, and M. Serrano, Tumour biology: senescence in premalignant tumours. Nature Vol. 436, pp642, 2005.