Gene Expression Profile of Proton Beam Irradiated Breast Cancer Stem Cells

Myung-Hwan Jung*, Jeong Chan Park

Korea Multi-purpose Accelerator Complex, Korea Atomic Energy Research Institute, Gyeong-ju 780-904
**Corresponding author: jungmh80@kaeri.re.kr*

1. Introduction

Cancer stem cells (CSCs) possess characteristics associated with normal stem cells: They can self-replicate (self-renewal) and differentiate into multiple cell types. Furthermore, they are resistant to conventional radiotherapy, such as photon beam irradiation, and chemotherapy owing to high DNA repair ability and oncogene overexpression. However, the mechanisms regulating CSC radio-resistance, including to proton beam, remain unclear.

Breast cancer stem cells (BCSCs) were first detected by Al-Hajj's group. They showed that a subset of cells expressing CD44 with weak or no CD24 expression could establish new tumors in xenograft mice. Recently, BCSC-targeting therapies have been evaluated by numerous groups. Strategies include targeting BCSC self-renewal, indirectly targeting the microenvironment, and directly killing BCSCs by chemical agents that induce differentiation, immunotherapy, and oncolytic viruses. However, the mechanisms regulating CSC radio-resistance, particularly proton beam resistance, remain unclear.

The identification of CSC-related gene expression patterns would make up offer data for better understanding CSCs properties. In this study we investigated the gene expression profile of BCSCs isolation from MCF-7 cell line.

2. Methods and Results

2.1 BCSCs isolation and pulsed proton beam irradiation

We were isolated BSCSs using fluorechrome-activated cell sorting (FACS) system. MCF-7 and MDA-MB-231 cells (1 × 107 cells/ml) were maintained in phosphate buffed saline (PBS) containing 0.5% bovine serum albumin (BSA). Fluorochrome-conjugated monoclonal antibodies against FITC-CD44 and PE-conjugated CD24 antibodies (BD Bioscience, Franklin Lakes, NJ, USA), or their respective IgG controls, were added to the cell suspension according to the manufacturer's protocol and incubated at room temperature in the dark for 0.5 to 1 h. The labeled cells were washed with wash buffer, then sorted on a FACS Jazz (BD Biosciences) to obtain CD44+/CD24-/low cells. (fig .1)

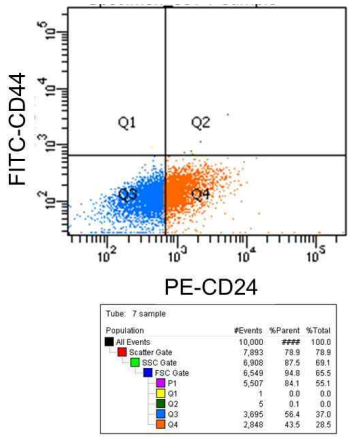

Fig. 1. The Result of BCSCs isolation by using fluorescence-activated cell sorting.

Cells were irradiated with pulsed proton beams at the Korea Multi-purpose Accelerator Complex (KOMAC). Protons (45 MeV) were produced with a spread Bragg peak using a ridge filter type modulator. Cells were irradiated with a single dose (1 to 12 Gy) of protons. The average dose rate was about 1 Gy/pulse. To monitor the dose, we used radio-chromic film (EBT-3, Ashland, Dublin, OH, USA). Attached cells were irradiated in a 96-well plate or T-25 flask filled with medium and placed on a beam stage. Plates were oriented so that the growth surface was orthogonal to the horizontal beam entering the plate bottom.

2.2 BCSCs are resistant to pulsed proton beam irradiation through MAPK phosphorylation.

We isolated BCSCs from the breast cancer cell lines MCF-7 which expressed the characteristic breast BCSC membrane protein markers $CD44^{+}/CD24^{-/low}$, and irradiated the BCSCs with pulsed proton beams. We confirmed that BCSCs are resistant to pulsed proton beams (fig. 2) and showed that treatment with p38 and ERK inhibitors reduced CSC radio-resistance. Based on these results, BCSC radio-resistance can be reduced during proton beam therapy by co-treatment with ERK1/2 or p38 inhibitors (fig. 3).

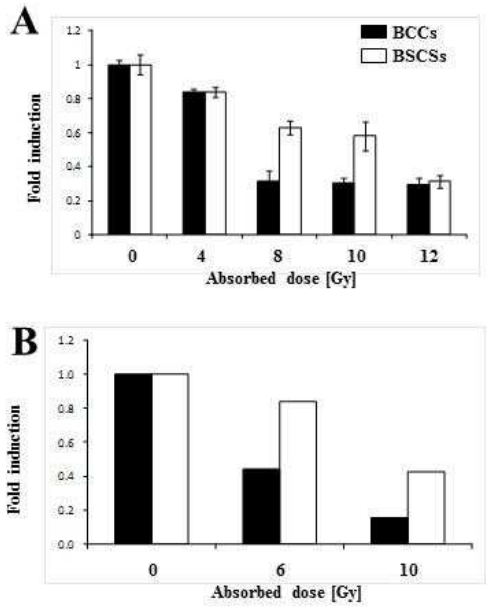

Fig. 2. Cell death following BCSCs and breast cancer cells (BCSs) pulsed proton beam irradiation. The results of (A) MTT assay and (B) colony formation assay of MCF-7cell death after pulsed proton beam irradiation.

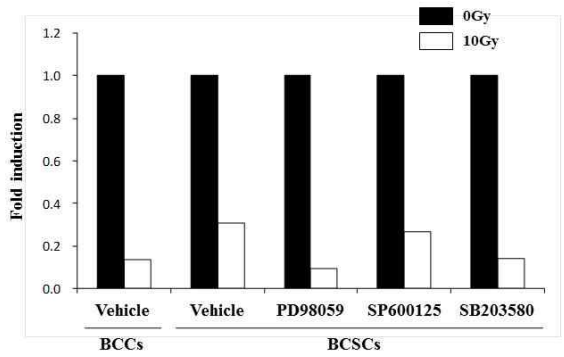

Fig. 3. Effect of MAPK inhibitors on survival an after pulsed proton beam irradiation in BCSCs.

2.3 Microarray analysis

Total mRNA was extracted from proton beam irradiated or non-irradiated MCF-7 cells using TRIZOL reagent method (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Microarray analysis was performed by Macrogen Inc. Microarray results are currently being analyzed. Analysis data will be presented at the meeting.

3. Conclusions

In the study, we showed that BCSCs are resistant to pulsed proton beam irradiation and that MAPK inhibitors specifically targeting ERK1/2 or p38 MAPK could overcome this radio-resistance (Fig. 2 and 3). Reducing BCSC resistance to pulsed proton beams is essential to improve therapeutic efficacy and decrease the 5-year recurrence rate. In this respect, the information of the level of gene expression patterns in BCSCs is attractive for understanding molecular mechanisms of radio-resistance of BCSCs.

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REFERENCES

[1] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison and M.F. Clarke, Proc Natl Acad Sci USA 100, 3983 (2003)
[2] M. Colleoni, G. Viale, D. Zahriehm G. Pruneri, O. Gentilini, P. Veronesi, R.D. Gelber, G. Curigliano, R. Torrisi, A. Luinim M. Intra, V. Galimberti, G. Renne, F. Nole, G. Peruzzotti and A. Goldhirsch, Clin Cancer Res. 10(19), 6622 (2004)
[3] J.E. Dick. Nature 423, 231 (2003).
[4] M. Diehn, R.W. Cho, N.A. Lobo, T. Kalisky, M.J. Dorie, A.N. Kulp, D. Qian, J.S. Lam, L.E. Ailles, M. Wong, B. Joshua, M.J. Kaplan, I. Wapnir, F.M. Dirbas, G. Somlo, C. Garberoglio, B. Paz, J. Shen, S.K. Lau, S.R. Quake, J.M. Brown, I.L. Weissman and M.F. larke. Nature. 458, 780 (2009)
[5] H. Korkaya, A. Paulson and F. Iovino. Oncogene 27, 6120 (2008)
[6] R.K.V. Kochhar, H. Bejjanki and G. Caldito, J Clin Oncol. 23, 6s (2005)
[7] M. Aleskandaran, Daniela Caracappa, C.C. Nolan, R. D. Macmillan, I.O. Ellis, E.A. Rakha, and A.R. Green. Breast Cancer Res Treat. 150, 81 (2015)