Identification of Radiation Exposure Biomarkers in C57BL/6 Mice

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1. Introduction

Variations in the radiation response in healthy people and human tissue and cells have also been described and may be important in predicting the potentially harmful effects of environmental, accidental, or therapeutic radiation exposure [1-3]. There is a need for a reliable method for quick and accurate measurement of radiation damage not only for use in a restricted number of individuals but also for public health purpose, especially for persons who exposed for therapeutic or diagnostic purpose. Radiation induced damage is dependent mainly on genetic damage, and thus far only cytogenetic approaches have been used to evaluate individual risk. Previously, we analyzed genes which have been reported to be overexpressed in human peripheral blood lymphocytes [4] and radiation inducible genes which were detected only in lung, spleen and intestine, but not in heart and brain were identified [5]. In this paper, we further elucidated that these genes were possibly candidates as blood biomakers for radiation exposure, especially local exposure for therapeutic or diagnostic purpose.

2. Methods

Animals

Female C57BL/6 mice, 6-7 weeks old, were purchased from Charles River Japan Inc. and were kept in clean conventional environment.

Irradiation

Whole body irradiation, 1 Gy was performed by using 137Cs gamma-ray source (Atomic Energy of Canada, Ltd., Ontario, Canada) with dose rate of 3.81 Gy/min.For the local region irradiation, animals were first anesthetized and localized in the radiation field. Then mice were irradiated with 1 Gy (190 cGy/min) on the thoracic, abdominal and lateral region following each experimental design. Sham exposed mice were used as control mice.

Quantitative real time PCR

Real-time PCR analysis was performed using a DNA Engine2.OPTICON(MJ Reserch) and the LightCycler-FastStart DNA Master SYBR Green I mix (Roche). 3'-locked nucleic acid (LNA) primers were synthesized by Proligo. Reactions were performed in a final volume of 15 l, adjusted to 4 mM MgCl2 and containing 500 nM each of primer and 2 l of DNA template. The real-time PCR cycling conditions wereas follows: 94C for 5 min, followed by 32 cycles for 1 min at 94C, 1 min at 52C and 2 min at 72C followed by fluorescence measurement. The polymerization temperature was set to 68C to allow accurate fluorescence measurements because of the low-melting temperature of the PCR products analyzed. Following PCR, a thermal melt profile was performed for amplicon identification. To determine the Ct, the threshold level of fluorescence was set manually in the early phase of the PCR amplification.

3. Results

We examined platelet membrane binding protein, protein tyrosine kinase, silalyltransferase and Cu/Zn SOD expressionsin blood lymphocytes of C57BL6 mice after 1 Gy whole body radiation. RT-PCR analysis indicated that these gene expressions were increasedat 1 day after radiation, which lasted until 3 days (Fig. 1A).

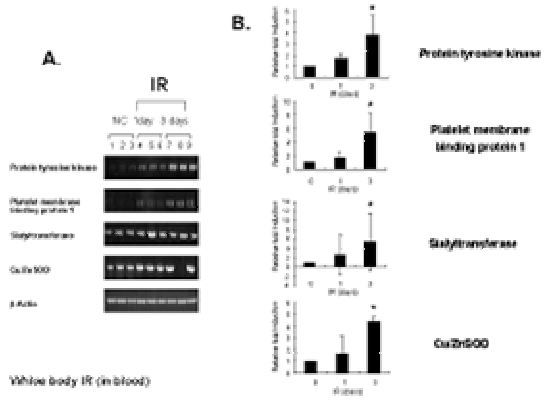

Figure 1. Expression of platelet membrane binding protein, protein tyrosine kinase, silalyltransferase and Cu/Zn SOD in blood lymphocytes of mice exposed to whole body radiation (1Gy). * Significantly different from the control at $p < 0.05$.

Quantification using real time PCR analysis in blood lymphocytes also confirmed that expression of these 4 genes was increased by 1 Gy whole body radiation (Fig. 1B).

When these genes were examined in blood lymphocytes at 1 or 3 days after radiation, when local irradiationto lung, spleen or intestine was performed, RT-PCR analysis of platelet membrane binding protein, protein tyrosine kinase, sialyltransferase and Cu/Zn SOD was shown that local irradiation to lung, spleen or intestine increased mRNAs of these genes in blood lymphocytes (Fig. 2A). Furthermore, quantification of these genes by real time RT-PCR also suggested that local irradiation increased gene expressions of platelet membrane binding protein, protein tyrosine kinase, sialyltransferase and Cu/Zn SOD (Fig. 2B).

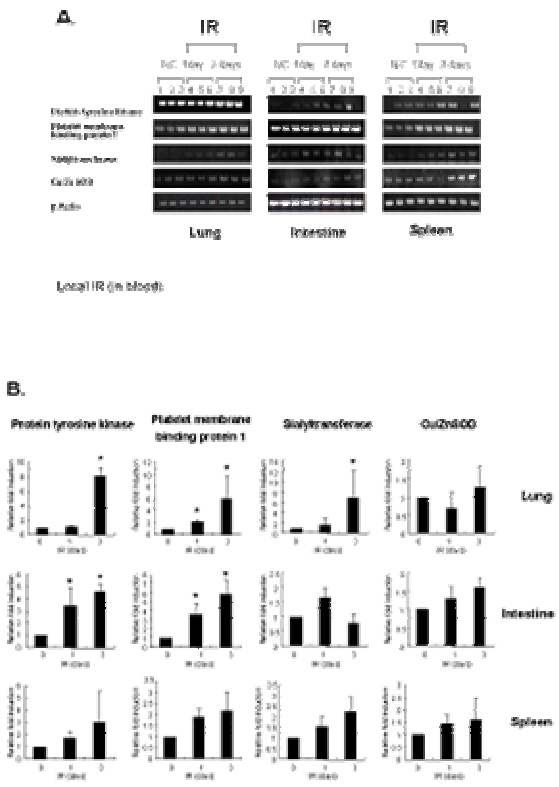

Figure 2. Increased gene expressions of radiation responsible genes in blood lymphocytes of locally irradiated mice. * denotes statistical siginficance compared with normal control ($p<0.05$).

4. Conclusion

When we examined these genes in the blood after whole body irradiation and local irradiation, 1Gy radiation induced these genes at 1 or 3 days after radiation exposure. From our data, platelet membrane binding protein, protein tyrosine kinase, silalyltransferase and Cu/Zn SOD might be candidattes for biomarkers after radiation exposure accidentally or therapeutic purposed and further experiments to elucidate the correlation between expression levels and prognostic effects after radiation, will be needed.

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