Identification of Radiation Response Organ Specific Proteins by Proteomics

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Introduction

Technologies using radiation are continuously developing in industry and medical diagnosis and therapy. We are kept in risk of exposure to radiation through occupational, medical, environmental, accidental, and other sources. We need biological indicators to detect radiation exposure. The effects of ionizing radiation on various tissue has been extensively investigated with respect to changes in histopathological and *in vitro* alterations. The damaging cellular and molecular effects of radiation exposures such as gene mutation, chromosomal abberration, cellular transformation, cell death and carcinogenesis has been well documented. However, these methods have practical limitations. Several attempts have been made to improve dosimetry using hematological, biochemical, immunologic, and cytogenetic endpoints.

In a pervious study, we performed gene profiling using microarray and confirmed gene expressions using RT-PCR in whole body irradiated or locally irradiated organs including the brain, lung, heart, intestine and spleen to identify possible organ specific candidate biomarkers for radiation exposure. [1-2].

Proteomics analysis is currently considered to be a powerful tool for global evaluation of protein expression, and proteomics has been widely applied in analysis or investigation. To characterize radiation response proteins in specific organs, we used 2-DE methods to detect difference between the control and irradiated proteome.

Materials and Methods

C57BL/6 mice (6-7 weeks old) were purchased from SLC (Hamamatsu, Japan). Animals divided randomly to two groups as normal control and irradiated group. The irradiation was carried out at 3.0 Gy/min for 20 seconds with whole body dose of 1 Gy. At 24h after irradiation, brain, lung, spleen, intestine, and bone marrow were obtained from each mouse. The bone marrow was harvested from each mouse by flushing the femoral shafts with cold Hank's balanced salt solution (HBSS). The bone marrow preparations from 5 mice were pooled and immediately centrifuged at 1300 rpm × 5 min at 4℃, removed supernatant, and then added RBC lysis buffer to cell pellets. Organs and bone marrow cells were stored in liquid nitrogen. The extraction of total

proteins from brain, lung, spleen, intestine, and bone marrow was performed, the proteins were separated with large gel 2-DE technique using 18 cm strip IEF and 9~17% acrylamide gels. The proteins patterns were evaluated with 2D gel image analysis software (Image master Platinum 5, GE Healthcare). Common spots from different two gel per irradiated or unirradiated organs were selected and compared protein expression level between normal control and 1 Gy irradiated organ samples.

Results and Discussion

Using a proteomic approach involving 2-DE, we have obtained proteomic characterization of radiation response in different organs. The common spots detected on paired gels of each organ were 997 for brain, 508 for lung, 1050 for intestine, 977 for spleen and 874 for bone marrow. We selected relatively up- or down-regulated protein spots (ratio $_{irradiation/control} \geq 2$) and Table 1 shows the number of spots increased or decreased proteins in their expression levels between normal and irradiated organs.

Table 1. Protein expression patterns in different organs by irradiation

	Total spot	up >2 folds	Down 2> folds
Brain	997	41	22
Lung	508	12	54
Intestine	1050	21	47
Spleen	977	27	28
Bone marrow	874	15	76

Conclusion

In the present study we investigated altered protein patterns in specific organs by radiation exposure. The proteomic approach makes an important contribution to characterizing radiation response proteome. We anticipate continued development of new technologies and procedures in the detection of radiation exposure.

Reference

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