Proteomics of Protein Expression Profiling in Tissues with Different Radiosensitivity

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1. Introduction

Ionizing radiation activates multiple signaling pathways, resulting in diverse stress responses including apoptosis, cell cycle arrest, and gene induction [1,2]. Liver tissue is known to be rather resistant to radiation while a spleen tissue is highly radiosentitive [3,4]. Our purpose was to compare radioresponse in liver and spleen following exposure to radiation to further investigate the differentially protein expression profile in radiosensitive and radioresistant tissues.

2. Methods and Results

Liver and spleen tissues in C3H/HeJ mice, after 10 Gy radiation treatments, were analyzed by 2-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) [4]. Tissue samples were stained with hematoxylin-eosin and terminal deoxynucleotidyl transferase-mediated dUTP Nick End Labeling (TUNEL) assay [5].

2.1 Analysis of Apoptosis

After 10 Gy of irradiation, the levels of apoptosis were lower liver than spleen ($p < 0.05$). The peak level of induced apoptosis was 35.3 ± 1.7% at 8h in spleen. In liver, we detected a weak signal after irradiation and the level of apoptosis was 0.6 ± 0.2%. This level of apoptosis appeared to vary in a tissues-specific manner.

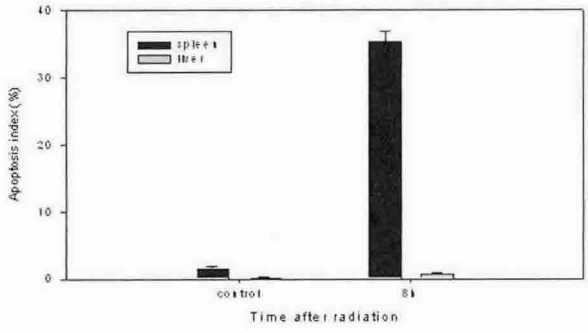

Figure 1. Apoptosis index of liver and spleen after radiation treatment were shown.Apoptosis index is percent number of apoptic body per 1000 nuclei.

2.2 Proteomic Analysis of the Irradiated Livers Proteins

To study the different proteins involved in ionizing radiation, we applied 2-DE to analyze the proteomics alteration of mouse tissues. Image analysis of 2-DE gels revealed that averages of 800 protein spots were detected and localized in pI 3 - 10 and molecular mass range 10 - 100 kDa. Comparison of these relatively abundant proteins on 2-DE gels using PDQUEST program revealed that protein spots were changed with their expression levels more than three fold in irradiated tissue. Both the pI and MW of each identified protein were determined by comparison to the standard 2-DE gels of the Swiss-2D PAGE database (http://kr.ExPaSy.org.). Those proteins were identified by peptide mass fingerprinting using MALDI-TOF mass spectrometry. Searching the MS - Fit database identified the peptide mass data.

At least twenty-eight proteins showed a significant quantitative alteration following radiation in liver. The increased proteins include cytochrome c, glutathione S transferase Pi (GSTP), carbonic anhydrase, NADH dehydrogenase and peroxiredoxin VI (Prx VI), whereas proteins such as protocadherin, phosphatidyl-ethanolamine and ras relative protein decreased after radiation (Table 1).

Since ROS has been known as key molecules in radiation–induced cellular injury, our 2-DE analysis concentrated on ROS metabolism regulating proteins. Especially, the expression level of Prx VI and cytochrome c was upregulated in the irradiated liver tissue ($P<0.05$). Partial 2-DE images for Prx VI and cytochrome c are shown in Fig. 1.

2.3 Proteomic Analysis of the Irradiated Spleen Proteins

Apoptosis index, the level of spleen was higher than liver after radiation. A proteomics results were showed that the increased proteins in irradiated spleen were more abundant than irradiated liver proteins. About 60 proteins significantly changed in level of protein patterns. The identified proteins were categorized as, signal transduction, apoptosis, cytokine, ca signal related protein, stress-related protein, cytoskeletal regulation, ROS metabolism, and others. Since radiation induced apoptosis is important factor in cellular response of normal tissues and tumor formation. Apoptosis proteins such as cytochrome c, bcl212 protein and tumor necrosis factor inducible protein were upregulated in the irradiated liver tissue (Table 2). Partial 2-DE images for Bcl-2 and cytochrome c are shown in Fig. 2. Also, the expression levels of the stress related proteins including Riken cDNA, stress-induced phosphoprotein, rick protein, delta–1-pyrroline–5-carboxylate and heat shock proteins were increased at 8 h after radiation (Table 1).

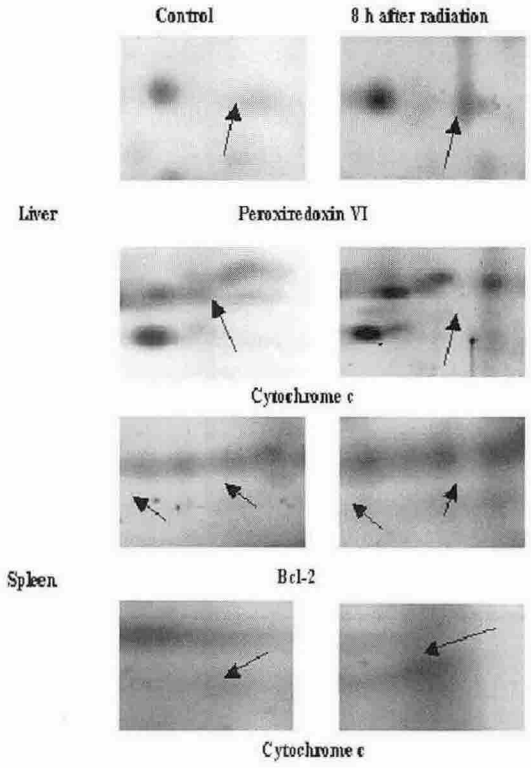

Figure 2. Partial 2-DE images for cytochrome c, Bcl-2 and peroxiredoxin VI are shown in liver and spleen tissues.

Table 1. Oxidative stress-related related proteins expression in liver and spleen after radiation

Protein Name	Spleen	Liver
	Control- RT	Control –RT
Gluthathione S transferase P1	-	Up
Gluthathione S transferase P2	-	Up
Peroxiredoxin IV	-	Up
NADH dehydrogenase	-	Up
Carbonic anhydrase	-	Up
RIKEN cDNA	Up	Up
Stress-induced phosphoprotein 1	Up	-
Rick protein	Up	-
Delta-1-pyrroline-5-carboxylate dehydrogenase	Up	-
Heat shock protein 1A	Up	-

Up represent: Up-regulated: 3 fold increased after radiation

Table 2. Apoptosis related proteins expression in liver and spleen after radiation

Protein name	Spleen ConRT	Liver ConRT	No. isoforms
Cytochrome c oxidase	Up	Up	
M-calpain	Up	-	
Bcl-2- related protein A1	Up	-	
CD59A glycoprotein precusor	Up	-	
Bcl 2l2 protein	Up	-	2
Iodothyronine deiodinase	Up	-	
Fas antigen	Up	-	
Tumor necrosis factor-inducible protein TSG-6 precursor	Up	-	

Up represent: Up-regulated: 3 fold increased after radiation

3. Conclusion

The levels of radiation-induced apoptosis were lower in the liver than the spleen tissues, which seemed to be related to the level of apoptosis protein expression due to the radiation in the liver and spleen tissues. In liver, proteomics result suggests that antioxidant enzymes such as Prx VI play important roles in this radio–susceptibility. Also, spleen proteomics results suggest that cytochrome c, bcl-2 protein and tumor necrosis may play an important role in radiation–induced apoptosis.

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