Proteome analysis of Radiation-induced pulmonary fibrosis

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1. Introduction

Pulmonary fibrosis is perhaps the most universal late effect of organ damage after both chemical insult and irradiation in the treatment of lung cancer. The use chemotherapy and radiation therapy, alone or combined, can be associated with clinically significant pulmonary toxicity, which leads to pneumonia and pulmonary fibrosis. It is also reported that about 100,000 people in the United States are suffered from pulmonary fibrosis [1]. Therefore, pulmonary fibrosis will be more focused by medicinal researchers. Because current therapies, aimed at inhibiting pulmonary inflammation that often precedes fibrosis, are effective only in a minority of suffered patients, novel therapeutic methods are highly needed [2,3]. Some researchers have used bleomycin-induced pulmonary fibrosis as a basis for looking at the molecular mechanisms of fibrosis [4,5], and total gene expression was monitored using genomics method [4]. However, radiation-induced pulmonary fibrosis has not been fully focused and investigated. Here, we have analyzed changes in gene expression in response to γ-irradiation by using proteomic analysis.

2. Materials and Methods

2.1 Induction of Radiation-induced pulmonary fibrosis

Five-week old female mice of the C57BL/6 were chosen for this study because of their known pulmonary radiosensitivity leading to severe pulmonary fibrosis. The mice were anesthetized with solution containing ketamine (1mg/ml) and rompun (1ml/ml). The thoraces were irradiated using a ^{60}Co γ-ray source operated at a dose rate of about 0.5 Gy/min. 25 Gy were given to the local thoraces. The mice were placed such that the thoraces were in the field defined by lead collimators. Following radiation, the mice were maintained ten per cage in pathogen-free rooms and supplied with standard laboratory chow and water. Age-matched controls were maintained under the same conditions. Treated and control mice were killed by cervical dislocation at 21 weeks after irradiation.

2.2 Histological analysis

The pieces of murine lung were fixed in buffered 10% formalin for 72 hr, embedded in paraffin and stained with hematoxylin-eosin. The stained sections were evaluated by light microscopy.

2.3 Hydroxyproline assay

The lyophilized tissue sample was homogenized thoroughly in distilled water using a polytron homogenizer (Biospec Products, Tissue-Tearer™). Test samples were hydrolyzed in alkali. The hydrolyzed samples were then mixed with a buffered chloramine-T reagent, and the oxidation was allowed to proceed for 25 min at room temperature. The chromophore was then developed with the addition of Ehrlich's reagent, and the absorbance of reddish purple complex was measured at 550 nm using spectrometer.

2.4 2-D PAGE

For all buffers, high quality, freshly dispensed Milli-Q purified water was used. Lung tissues were suspended in homogenization buffer (5mM $NaPO_4$ (pH7.4), 5 mM EDTA, 0.32M sucrose, 1mM 2-ME, and protease inhibitor cocktail (Complete, Roche)) using polytron homogenizer. Homogenates were then centrifuged at 3500 rpm for 10 min to remove tissue and cell debris. The supernatants were then taken and centrifuged at 12000g for 45 min. The final supernatants were obtained. For desalting step, the final supernatants and sample buffer (40 mM Tris, 7 M urea, 2 M thiourea, 100 mM DTT, 2% CHAPS, and protease inhibitor cocktail) were mixed respectively into Centricon YM-3 (Millipore), which was centrifuged at 3500 rpm at 12℃ for 2 hr. This step was repeated 6 times to eliminate salt and lipid completely in lung sample. The protein concentration of the supernatants was determined by Bradford assay method and 40mg of total lung protein was used for each electrophoresis. Aliquots of lung proteins in sample buffer were applied onto immobilized pH 3 to pH 10 linear gradient strips. The second dimension was performed on 10% to 16% linear gradient polyacrylamide gels (24cm x 18cm x 1.5mm) at 40mA per gel constant current for approximately 6 hours. After protein fixation for 12 h, gels were stained with silver nitrate and scanned in a Bio-Rad G710 densitometer. Gel images were converted into electronic files, which were then analyzed with PDQuest computer software (Bio-rad).

3. Results

In order to establish the RIF mouse model, several doses of irradiation and adequate time after irradiation were tested. At each point, the degree of RIF was evaluated by H/E staining and hydroxyproline assay. In our experiments, RIF was induced by 25Gy at 21weeks

after irradiation displayed early-phase pulmonary fibrosis by eosin staining and hydroxyproline assay.

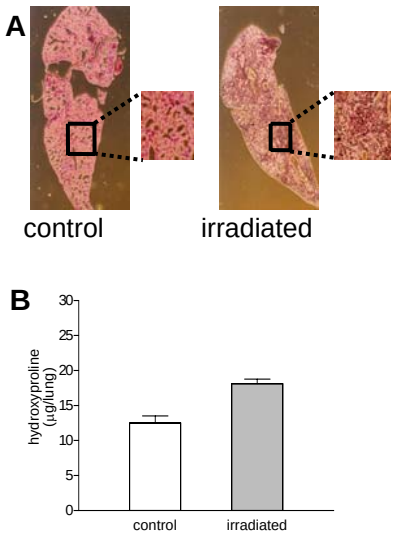

Figure 1. Fibrosis confirmation using H/E staining (A) and hydroxyproline assay (B).

Proteomic analysis was performed with mouse lung tissues isolated from age-matched control and pulmonary fibrosis tissues and analyzed by 2DE. More than 1,000 protein spots were detected on the gels and localized in the ranges of pI 3 to 10 and relative molecular mass 10 to 100 kDa. Computer-assisted comparative analysis of the respective silver staining spot patterns of the 8 paired samples showed that about 60 proteins' expression level varied in total protein samples from RIF. Unfortunately, some protein spots were not identified using Maldi-tof analysis. However, we could discover that α 1-protease inhibitor and galectin-1 in radiation-induced pulmonary fibrosis tissue were found to be significantly increased.

4. Conclusion

The presented study firstly showed that α 1-protease inhibitor and galectin-1 were expressed at significantly high levels in the early-phase of radation-induced fibrotic lungs. Based on the other researcher's results, we suggested that overexpression of α 1-protease inhibitor [6] and galectin-1 [7] can protect lung tissue from radiation-induced pulmonary fibrosis. Further experiments should be performed to investigate whether the α 1-protease inhibitor and galectin-1 can be detected in blood or BAL fluid from RIF patients and could be applied as a drug target or disease marker.

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