Overexpression of matrix metalloproteinase-12 (MMP-12) correlates with radiation-induced lung fibrosis

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1. Introduction

MMPs are a family of more than 20 secreted or transmembraneproteins that are capable of digesting extracellular matrix and basement membrane components under physiologic conditions [1]. According to their substrate specificity and structure, MMPs are classified into five subgroups: collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, MMP-11), as well as metalloelastase (MMP-12), the membrane-type MMPs (MMP14, MMP15), and other MMPS (e.g., MMP-19, and MMP20) [2]. MMP-12 (matrix metalloproteinase12), also known as macrophage metalloelastase, was first identified as an elastolytic metalloproteinase secreted by inflammatory macrophages 30 years ago [3]. MMP-12 degrades extracellular matrix (ECM) components to facilitate tissue remodeling. It can degrade elastin and other substrates, such as type IV collagen, fibronectin, laminin, gelatin, vitronectin, entactin, heparin, and chondroitin sulfates [4, 5]. In the lung, MMP-12 is identified in alveolar macrophages of cigarette smokers as an elastolytic MMP [6]. Inactivation of the MMP-12 gene in knockout mice demonstrates a critical role of MMP-12 in smoking-induced chronic obstructive pulmonary disease (COPD) [7]. In addition, MMP-12 overexpression in alveolar type II epithelial cells directly triggered lung tumorigenesis as a result of pulmonary inflammation [8]. However, the effect of MMP-12 by irradiation in lung still remains largely unknown. The aim of the present study was to investigate the effects of MMP-12 by radiation in lung, so we evaluate that MMP-12 expression pattern in normal lung tissue and cancer cell following radiation.

2. Methods and Results

2.1 Experimental design

Cells were exposed to γ-ray with a ^{137}Cs γ-ray source (Atomic Energy of Canada, Mississauga, Ontario, Canada). Cells were harvested at 0 and 24 hr after 10 Gy and 20 Gy radiation. Mice were randomly divided into seven groups: A) normal control group; B) 1 days after 25 Gy radiation group; C) 3 days after 25 Gy radiation group; D) 5 days after 25 Gy radiation group; E) 7 days after 25 Gy radiation group; F) 14 days after 25 G y radiation group; G) 21 days after 25 Gy radiation group. Mice were exposed to radiation using X-RAD 320 (Precision X-ray, Inc., USA) at 250 kV, 10 mA with 42 mm aluminum added filtration. The mice imaged with X-ray scanning before whole lung irradiation. Animals were sacrificed at 1, 3, 5, 7. 14 and 21 days after whole lung radiation. Lungs were immediately fixed in formalin, and paraffin-embedded for morphometric analysis and immunohistochemical staining.

2.2 Cell culture and reagents

Human lung cancer A549 cells were obtained from ATCC (USA). A549 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS, Lonza, USA), 1% antibiotic and antimycotic (Gibco, USA), A549 cells were grown at 37 ºC in 5% CO_2.

2.3 Animals

Male BALB/c mice were obtained from Orient Inc. (Seoul, South Korea) at 7 weeks of ages (average body weight, 18.2 ± 2.1 g) and held for 1 week prior to experiments. Animals were kept under SPF conditions with free access to water and food. The studies were carried out under the guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the Korea Institute Radiological and Medical Sciences (KIRAMS).

2.4 Western blot analysis

For western blot analysis of MMP-12, cells were collected and lysed with RIPA buffer containing Complete Protease Inhibitor Cocktail (Roche Diagnostics, Indianapolis, IN, USA), and protein concentration was determined by protein assay (Bio-Rad, Hercules, CA, USA). Western blot analysis was performed for MMP-12 using the following antibodies; MMP-12 Polyclonal Antibody (Santa Cruz Biotech, CA, USA), SMA-a Polyclonal Antibody (Abcam, MA, USA) and β-actin (Sigma, St. Louis, MO, USA).

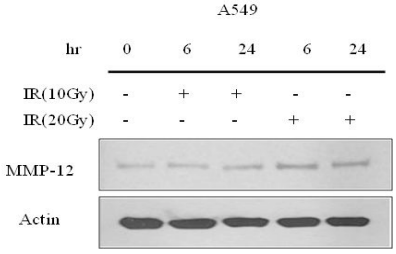

Figure 1. Changes of MMP-12 protein levels during different time and radiation dose points in A549 cell line by radiation.

Radiation did alter protein levels of MMP-12 in human lung cancer cell line. MMP12 was significantly up-regulated at 20 Gy radiation but not at 10 Gy radiation.

2.5 Immunohistochemistry

Sections were taken at 3 ㎛ , dewaxed and then rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked with 0.3% H_2O_2 in absolute methanol for 15 min at room temperature. For antigen retrieval, sections were placed in a citric buffer (pH 6.0) which was heated in an autocleave for 20 min. Non-specific immunoglobin binding was prevented by incubating section in blocking solution for 30 min. Section were incubated at room temperature with diluted anti-MMP-12 (Santa Cruz Biotech, CA, USA) or anti-αSMA (Abcam, MA, USA), and washed with PBS containing 0.1% Triton X-100 (2 x 4 min). Incubation with the corresponding secondary antibody and the peroxidase antiperoxidase (PAP) complex was carried out for 30 min at room temperature. The immunoreactive sites were visualized using 3,3'-diaminobenzidine (0.1%), and hydrogen peroxide solution (0.03%). Hematoxylin was used as the counter-stain.

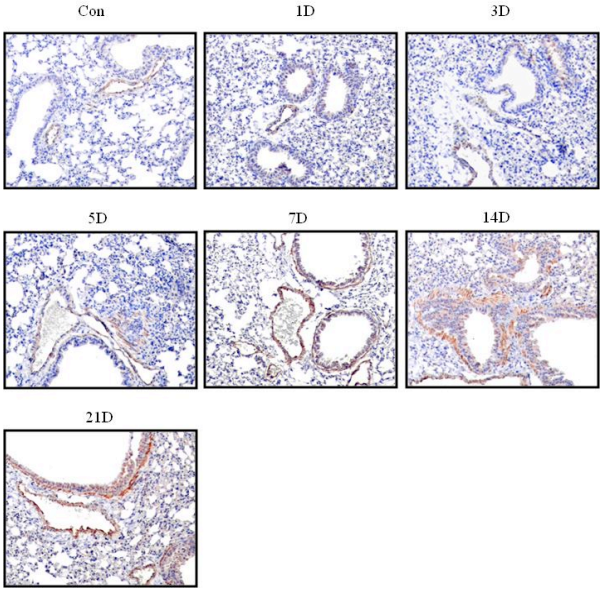

Figure 2. Expression pattern of MMP-12 during different time points in normal lung tissue following whole lung radiation. (magnification; × 100)

2.6 Masson's Trichrome staining

Sections were taken at 3 ㎛ , dewaxed and then rehydrated. After sections were incubated in Bouin`s solution for 1 hr at 56 ℃, washed in running tap water. The sections stained in iron haematoxylin for 1 min, washed in running tap water for 15 min. And Sections were stained with biebrich scarlet-acid fuchsin solution for 15 min and treated with phostotungstic/ phoshomolybdic acid for 15 min. Then, sections stained with aniline blue solution for 10 min, washed in distilled water, dehydrated, and mounted.

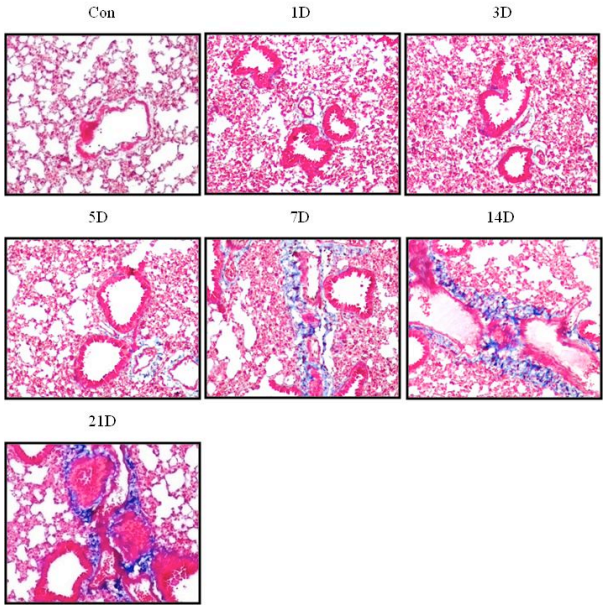

Figure 3. Alteration of collagen (Blue) during indicated time points in normal lung tissue following whole lung radiation. (magnification; × 100)

In animal study, MMP-12 could be detected within 5 days, and significantly increased its expression until 21 days after radiation exposure. Correlation with MMP12 overexpression, collagen detected by Masson's trichrome staining and αSMA which are general markers for lung fibrosis were increased time dependently in the lung following radiation.

3. Conclusions

Radiation induced lung injury most commonly occurs as a result of radiation therapy administered to treat cancer. The present study demonstrates that MMP-12 was highly increased in the lung damaged by radiation Thus, MMP-12 might be of potential relevance as a clinically diagnostic tool and sensitive biomarker for radiation induced lung injury and fibrosis.

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