ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 39 (2015) 917–926

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

The inflammasome accelerates radiation-induced lung inflammation and fibrosis in mice

Sung-Hwa Sohn[a], *Ji Min Lee*[a], *Soojin Park*[b], *Hyun Yoo*[a], *Jeong Wook Kang*[a], *Dasom Shin*[b], *Kyung-Hwa Jung*[b], *Yun-Sil Lee*[c], *Jaeho Cho*[a,*], *Hyunsu Bae*[b,**]

[a] *Department of Radiation Oncology, Yonsei University College of Medicine, Seoul, South Korea*
[b] *Department of Physiology, College of Oriental Medicine, Kyung Hee University, Seoul, South Korea*
[c] *College of Pharmacy & Division of Life and Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, South Korea*

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form
24 February 2015
Accepted 27 February 2015
Available online 7 March 2015

Keywords:
Radiotherapy
Pneumonitis
Fibrosis
Inflammasome

ABSTRACT

Although lung inflammation and fibrosis are well-documented dose-limiting side effects of lung irradiation, the mechanisms underlying these pathologies are unknown. An improved mechanistic understanding of radiation-induced pneumonitis is a prerequisite for the development of more effective radiotherapy; this was the rationale for the current study. Mouse lungs were focally irradiated with 75 Gy. The numbers of neutrophils, lymphocytes, macrophages, and total cells in the bronchoalveolar lavage fluid were counted, and pro-inflammatory cytokine levels were measured. Histological analysis and immunohistochemical staining for Tgf-β1 and Cd68 (a macrophage-specific protein) was also performed. After irradiation, mice developed pneumonitis, and exhibited higher numbers of neutrophils, lymphocytes, eosinophils, macrophages, and total cells compared to controls. In addition, inflammasome (Nlrp3, and caspase 1, Il1a, and Il1β), adhesion molecule (Vcam1), and cytokine (Il6) genes were significantly upregulated in the IR group. Cd68 and Tgfb1 proteins were significantly increased after irradiation. Upregulation of Cd68 and Tgfb1 correlates with the onset of radiation-induced pneumonitis and fibrosis. In addition, radiation-induced pneumonitis and fibrosis are accompanied by upregulation of phenotypic markers of inflammasome activity. Our findings have implications for the onset and exacerbation of damage in normal lung tissue.

* *Corresponding author at*: Department of Radiation Oncology, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, South Korea. Tel.: +82 2 2228 8113; fax: +82 2 2227 7823.
** *Corresponding author at*: Department of Physiology, College of Oriental Medicine, Kyung Hee University, #1, Hoegi-dong, Dongdaemun-gu, Seoul 130-701, South Korea. Tel.: +82 2 961 9316.
E-mail addresses: jjhmd@yuhs.ac (J. Cho), hbae@khu.ac.kr (H. Bae).

http://dx.doi.org/10.1016/j.etap.2015.02.019
1382-6689/

1. Introduction

Radiotherapy is a standard treatment for patients with non-small cell lung cancer (NSCLC) (Pfister et al., 2004). Conventionally fractionated radiotherapy for NSCLC consists of 1.8–2.0 Gy fractions given once daily for 5 days each week to a total dose of 60 Gy or more (Mehta, 2005). Radiation oncologists search for procedures to eradicate tumors while sparing normal tissues. Nevertheless, the adverse effects of radiation in normal tissues that surround the tumor often preclude the application of curative radiation doses. These radiation-induced lung toxicities, which include the activation of the acute inflammatory response (pneumonitis) and late pulmonary fibrosis, are common. In particular, radiation pneumonitis is an increasingly prevalent cause of morbidity and mortality (Kong et al., 2005; Yamashita et al., 2007). While the precise mechanisms underlying this pathology are unknown, they may involve radiation damage to the alveolar epithelium and capillary endothelium, or an innate immune response to tissue inflammation and injury (Marks et al., 2003; Schaue and McBride, 2010; Tsoutsou and Koukourakis, 2006).

The innate immune system senses tissue damage that translates the information to other repair and defense systems in the body by stimulating angiogenesis, wound repair, and activating adaptive immunity (Demaria et al., 2010). Pathogen-associated molecular patterns (PAMPs) are recognized by molecules of the innate immune system termed "pattern recognition receptors" (PRRs) (Takeuchi and Akira, 2010). Damaged host cells can also activate PRRs by releasing danger-associated molecular patterns (DAMPs). These PRRs are expressed by cells at the front line of the host defense, which include macrophages, monocytes, dendritic cells, and epithelial cells (dos Santos et al., 2012). Within the PRR family, the nucleotide oligomerization domain-like receptors (NLRs) trigger a distinct defense mechanism by promoting the assembly of inflammasomes (Martinon et al., 2002). Activation of inflammasomes requires two signals: the first involves sensing of conserved PAMPs that induce expression of certain inflammasome components and cytokines, while the second is elicited upon disruption of cellular membranes or signaling pathways (Mariathasan and Monack, 2007). These events subsequently trigger activation of inflammasome/caspase-1 signaling, which is followed by IL1β and IL18 processing and secretion in dendritic cells, macrophages, and epithelial cells (Basak et al., 2005; Ghayur et al., 1997; Hitzler et al., 2012; Kim et al., 2013; Kostura et al., 1989; Yang et al., 2013). Although the pathogenesis of pulmonary diseases such as acute lung injury, pulmonary fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) has been extensively studied, the role of the inflammasome in events that link radiation-induced pneumonitis and fibrosis requires further definition. In this study, we selected an irradiated animal model to obtain an in-depth understanding of the molecular events associated with concomitant pneumonitis and fibrosis.

2. Materials and methods

2.1. Irradiation

Radiation was delivered to a small volume of the left lung by using an image-guided small-animal irradiator, the X-RAD 320 (Precision, North Branford, CT, USA). This irradiator is equipped with a collimator system composed of 3.5-cm thick copper to produce focal radiation beams, an imaging subsystem consisting of a fluorescent screen coupled to a charge-coupled-device camera, and a manually operated stage. The collimators generate a cone beam that ranges from 1 to 7 mm in diameter. The percentage depth doses were measured with GAFCHROMIC EBT2 film (Hong et al., 2014). To mimic clinical stereotactic ablative radiotherapy conditions by irradiating only a small tissue volume, we selected 3-mm collimators.

2.2. Mouse irradiation

All studies involving the use of mice were approved by the Yonsei University Medical School Animal Care and Use Committee. C57BL/6 female mice (8 weeks old, weighing 25 g) were purchased from Charles River Korea (Orient Bio, Seongnam, South Korea). The mice were housed ($n = 5$ in each cage) and allowed to acclimatize for 1 week (w) before treatment. The study was performed according to the ethical principles and guidelines established by the Yonsei University for the care and use of experimental animals. A single dose of 75 Gy was delivered to the left lung in a single fraction.

In our previous study, we found that a radiation dose of 75 Gy induced inflammation after 2 w. In this study, we evaluated dose-response relationships up to 2–3 w after 75-Gy radiation. The mice were randomly divided into nine groups (control, 2 w and 3 w after 75 Gy). During irradiation, the mice were anesthetized with an intraperitoneally administered mixture of 30 mg/kg of zoletil and 10 mg/kg of rompun. At 2 w or 3 w after 75 Gy, the mice were killed. Three mice were allocated per group, and the experiment was repeated twice.

2.3. Analysis of lung inflammatory cells

Phosphate-buffered saline (PBS) was gradually infused into the lungs and then withdrawn via a cannula that had been inserted into the trachea. The numbers of total and differential cells in the bronchoalveolar lavage (BAL) fluid were then determined using a hemocytometer. In addition, differential cell counts were conducted on slides that were prepared by cytocentrifugation and Diff-Quick staining. Approximately 500 cells were counted per slide. The BAL fluids were then centrifuged, and the supernatants were stored at −80 °C until further analysis.