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Naringenin Ameliorates Radiation-Induced Lung Injury by Lowering IL-1β Level[S]

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ABSTRACT

Radiation-induced lung injury (RILI) is the main complication of radiotherapy for thoracic malignancies. Since naringenin, a potent immune-modulator, has been found to relieve bleomycin-induced lung fibrosis by restoring the balance of disordered cytokines, we sought to determine whether naringenin would mitigate RILI and to investigate the underlying mechanism. Animals received fractionated irradiation in the thoracic area to induce RILI. Enzyme-linked immunosorbent assay and MILLIPLEX assays were used for serum and bronchoalveolar lavage fluid for cytokine analyses, hematoxylin and eosin staining for pathologic changes, and Masson trichrome staining for determination of lung fibrosis. Interleukin (IL)-1β was found significantly elevated after thoracic irradiation and it triggered production of profibrotic tumor growth factor β both in vivo and in vitro, suggesting the vital role of in IL-1β in the development of RILI. Furthermore, we found that naringenin was able to ameliorate RILI through downregulation of IL-1β and restoration of the homeostasis of inflammatory factors. Our results demonstrated that naringenin could serve as a potent immune-modulator to ameliorate RILI. More importantly, we suggest that a new complementary strategy of maintaining the homeostasis of inflammatory factors combined with radiation could improve the efficacy of thoracic radiotherapy.

Introduction

Radiotherapy is a critical component in the treatment of thoracic malignancies like esophageal, lung, and breast cancers (Atun et al., 2015), in which lungs inside the radiation field are exposed to potential injury. Radiation dose over 50 Gy may lead to the development of radiation-induced lung injury (RILI), a term that comprises early radiation pneumonitis and late radiation-induced pulmonary fibrosis (Rodrigues et al., 2004; Kubo et al., 2009; Cui et al., 2015). In particular, 13%–37% of lung cancer patients, nearly 20% of breast cancer patients, and around 15% of esophageal cancer patients who receive thoracic radiotherapy develop RILI. Radiation pneumonitis involves proinflammatory cytokine and chemokine production causing inflammatory immune-cell infiltration and destruction of lung parenchyma and stromal and epithelial cells (Schallenkamp et al., 2007). Moreover, radiation pneumonitis is known to progress to radiation-induced pulmonary fibrosis characterized by fibroblast proliferation, collagen accumulation, and destruction of the normal lung architecture (Tsoutsou and Koukourakis, 2006; Benveniste et al., 2013; Ding et al., 2013).

Transforming growth factor β (TGF-β) drives the most important events associated with radiation-induced pulmonary fibrosis (Tsoutsou and Koukourakis, 2006; Ding et al., 2013; Zhang et al., 2015). Therefore, therapies directed at regulating TGF-β downstream Smad activity or connective tissue growth factor would probably be effective for the treatment of radiation-induced pulmonary fibrosis. Before fibrogenesis, radiation pneumonitis involves multiple cytokines, exemplified by IL-1α, IL-1β, TNF-α, and IFN-γ (Mancini and Sonis, 2014). Thus, whether there exists a key cytokine upstream of TGF-β that is associated with both radiation pneumonitis and radiation pulmonary fibrosis remains an open question. Various stimuli, such as multiwalled carbon nanotubes, *Pseudomonas aeruginosa*, bleomycin, or irradiation can induce lung fibrosis, during which inflammasome is activated, indicating involvement of an IL-1 family member in the pathogenesis of lung fibrosis (Gasse et al., 2007; Wilson et al., 2010; Palomo et al., 2014; Sun et al., 2015; Zhang et al., 2016b). IL-1R1 deficiency protects against the onset of lung inflammation and fibrosis (Gasse et al., 2007), and exogenous IL-1β administration or IL-1β overexpression

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ABBREVIATIONS: BALF, bronchoalveolar lavage fluid; PCR, polymerase chain reaction; RILI, radiation-induced lung injury; TGF, transforming growth factor; SBE, Smad binding element.

result in acute inflammation and subsequent lung fibrosis in rodent models (Gasse et al., 2007; Wilson et al., 2010). Moreover, an increased level of IL-1β in the saliva of patients with head and neck cancer receiving radiotherapy has been reported (Bossi et al., 2016). So, we asked whether IL-1β is a key player in the development of RILI.

Our previous studies had demonstrated that naringenin, the predominant flavonoid in grapefruit, could ameliorate bleomycin-induced pulmonary fibrosis and reduce tumor lung metastasis in mice and prolong life span by subverting the immunosuppressive environment (Du et al., 2009). Naringenin also inhibited TGF-β secretion and signaling (Lou et al., 2012; Zhang et al., 2016a), Smad3 expression and phosphorylation (Liu et al., 2006), and lipopolysaccharide-induced TNF-α secretion (Jin et al., 2017). Those data prompted us to hypothesize that naringenin may be a potential adjuvant in combination with thoracic radiotherapy to enhance its efficiency and simultaneously attenuate RILI. In the present study, we demonstrated that IL-1β played a pivotal role in RILI, and naringenin remarkably protected against RILI by downregulating the level of IL-1β. More importantly, naringenin could maintain the homeostasis of radiation-induced inflammatory factors.

Materials and Methods

Mouse Radiation-Induced Lung Injury Model. Female BALB/c mice (8-week-old) were purchased from Vital River Laboratories (Beijing, China) and housed in the Animal Care Facility of the Institute of Biophysics, Chinese Academy of Sciences. Irradiation was performed with mice restrained in a customized lead-shield apparatus that allowed for selective irradiation of the lung area. Before irradiation, the mice were anesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg; MilliporeSigma, St. Louis, MO), and 8 Gy $\times$ 2F (two consecutive days) irradiation was delivered to the lungs (γ-ray from ^{60}Co source in College of Chemistry and Molecular Engineering, Peking University, 1.8 Gy/min). Dosimetry was determined with thermoluminescent dosimeters. Immediately after irradiation mice were removed from the equipment and housed in a climate- and light/dark-controlled environment. All mice were given ad libitum access to food and water. At designed time points (1, 7, 14, and 21 days after irradiation), bronchoalveolar lavage fluid (BALF) was collected for cytokine detection and lung tissues were collected for hematoxylin and eosin staining. The study was institutionally approved by the Institutional Animal Care and Use Committee, Institute of Biophysics, Chinese Academy of Sciences.

Rat Radiation-Induced Lung Injury Model. Female Wistar rats (7-week-old) were purchased from the experimental animal center of Hubei province, China, and housed in the animal care facility of Renmin Hospital of Wuhan University. Before irradiation, rats were anesthetized with isoflurane gas. Under the protection of a lead plate that allows selective irradiation of right lung area, 6 Gy $\times$ 5 f (five consecutive days) X-ray (Varian linear accelerator; Varian Medical Systems, Palo Alto, CA) was delivered to the right lungs to mimic clinical stereotactic ablative radiotherapy. Immediately after irradiation, rats were removed from the equipment and housed in a climate- and light/dark-controlled environment. All rats were given ad libitum access to food and water. At designed timepoint (3, 7, 15, and 30 days after irradiation), serum was collected for cytokine detection and lung tissues were collected for hematoxylin and eosin staining. The study was institutionally approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University.

IL-1β Neutralization and Naringenin Treatment in Mouse Radiation-Induced Lung Injury Model. In the mouse irradiation-induced lung injury model, 100 μg/mouse of anti-IL-1β antibody was given (i.p.) from the day mice received irradiation and following at a frequency of one injection every 3 days for a total of 10 times. In addition, 200 mg/kg of naringenin was orally administrated 3 days before irradiation for 10 days followed by 100 mg/kg of naringenin for another 3 weeks. Lung tissues were collected at 3 and 60 days after irradiation and fixed by formaldehyde for hematoxylin and eosin staining and BALF were collected at 7 days after irradiation for IL-1β detection. Anti-IL-β antibody (Clone B122) was purchased from Bio X Cell (Lebanon, NH), and naringenin was purchased by Topfond Pharmaceutical Co., Ltd. (Zhumadian, P.R. China).

Cytokines, Chemokines, and Growth Factor Analysis. After full exposure of the trachea, lungs were infused with 500 μl of prechilled phosphate-buffered saline, and BALF was collected by centrifugation (4°C, 500*g*, 5 minutes) according to a previously published protocol (Moro et al., 2015). Blood was collected and placed on ice for 2 hours, and serum was collected by centrifugation of blood samples (4°C, 2000*g*, 20 minutes). All the samples were stored at −80°C for further detection.

Cytokines, chemokines, and growth factors in rat serum were quantified using MILLIPLEX assays (MilliporeSigma, Burlington, MA). IL-1β (BioLegand, San Diego, CA) and TGF-β (Promega, Madison, WI) in mouse BALF and serum, or supernatant and cell lysate of L929, were measured by ELISA according to the manufacturer's instructions.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction. Total RNAs were isolated using TRIzol reagent (Invitrogen/Thermo Fisher Scientific, Waltham, MA). Reverse transcription polymerase chain reaction (PCR) was performed using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd., Beijing, P.R. China) according to the manufacturer's instructions. Quantitative real-time PCR was performed using TransSmart Green qPCR SuperMix UDG (TransGen) on an ABI 7300 (Thermo Fisher Scientific). The primers for matrix metallopeptidase 9 were 5′-CTGGACAGCCAGACACTAAAG-3′ and 5′-CTCGCGGCAAGTCTTCAGAG -3′; primers for β-actin were 5′-TGACGTTGACATCCGTAAAGACC-3′ and 5′-AAGGGTGTAAAACG-CAGCTCA-3′.

Cell Culture and Reporter Gene Assay. L929 fibroblasts (ATCC) were cultured (37°C incubator with 5% CO_2) in RPMI 1640 (Gibco/Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (HyClone/Thermo Fisher Scientific), penicillin (100 IU/ml; Beyotime Biotechnology, Jiangsu, China) and streptomycin (100 IU/ml; Beyotime). Smad binding element (SBE)-luc2 plasmid was a kind gift from Prof. X. Liu (Institute of Hydrobiology, Chinese Academy of Sciences) and was used as a probe to detect whether the TGF-β target genes were activated when transfected into recipient cells.

When cells reached 70% confluence, SBE-luc2 plasmids were transfected into L929 cells using Lipofectamine 2000 according to the manufacturer's instructions at the concentration of 2.5 μg per 10^5 cells. Thirty-six hours later, L929 cells were treated with 1 ng/ml IL-1β for 1 or 4 hours before the intensity of chemiluminescence was detected.

Statistical Analysis. Values were presented as mean $\pm$ S.D. Unpaired Student *t* test was used to make comparisons between two independent groups. All data were analyzed using GraphPad Prism 6, and P values <0.05 were considered statistically significant.

Results

Naringenin Alleviates Radiation-Induced Lung Injury. Our previous study had shown that naringenin, as a potent immune-modulator, ameliorated bleomycin-induced lung fibrosis by maintaining the homeostasis of cytokines (Du et al., 2009). Given the similarity of pulmonary pathogenic manifestations induced by bleomycin and irradiation, we speculated that naringenin may also alleviate radiation-induced lung injury. First, in a rat model, at days 3, 7, 15,

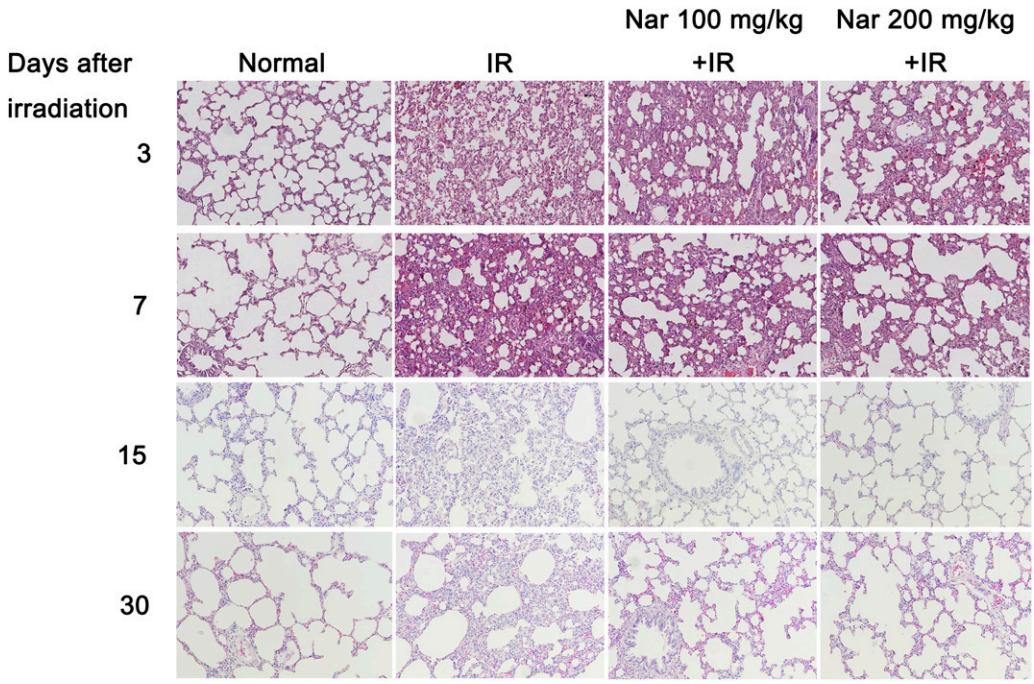

Fig. 1. Naringenin (Nar) ameliorates radiation-induced lung injury in rats. The right lungs of Wistar rats were exposed to 6 Gy × 5 f X-ray (IR). Daily 100 or 200 mg/kg of Nar (oral) was administered for 30 days starting at day 3 before IR. Lung tissues (n = 3 in each group) were collected at days 3, 7, 15, and 30 after IR for hematoxylin and eosin staining (original magnification, 200×).

and 30 after 6 Gy × 5 f thoracic irradiation procedure, we collected lung tissues for histologic analysis on pulmonary inflammation status. Hematoxylin and eosin staining showed that naringenin treatment effectively relieved the radiation-induced lung injury (Fig. 1). Interestingly, we also noticed that both the 100- and 200-mg/kg of naringenin were able to control early inflammatory cell infiltration, measured at day 3 after irradiation, whereas 100 mg/kg of naringenin was more effective for the amelioration of the thickening of alveolar septa than 200 mg/kg of naringenin, measured at day 30 after irradiation.

IL-1β Plays the Vital Role in Radiation-Induced Lung Injury. It has been reported that IL-1β and TGF-β are involved in the pathogenesis of lung injury under various insults (Kolb et al., 2001; Wilson et al., 2010; Wang et al., 2017), but how they are affected by thoracic irradiation has not been clearly elucidated. To this end we first used the rat RILI model as in Fig. 1, in which acute lung pneumonitis was observed as early as 3 days after 6 Gy × 5 F thoracic irradiation (Fig. 2B). Thus, we monitored the dynamic changes in IL-1β and TGF-β from day 3 after thoracic irradiation. At day 3 after irradiation, IL-1β slightly decreased but significantly increased and reached a peak at day 7 after irradiation, followed by a gradual decline within 30 days (Fig. 2C). In contrast, TGF-β still declined until day 7 after irradiation but recovered after 15 days post-irradiation (Fig. 2D). Irradiation may have been compromising both lung tissue and resident immune cells at day 3 after irradiation, resulting in a reduced production of IL-1β and TGF-β. However, irradiation-induced acute inflammation correlated with a subsequent increase in IL-1β and TGF-β at day 7 after irradiation.

To further confirm our findings, we employed wild-type BALB/c mice receiving 8 Gy × 2 F fractionated γ-ray irradiation to their whole lungs as another rodent model (Fig. 2F). As shown in Fig. 2G, irradiation also caused severe pneumonitis in mice, and IL-1β gradually elevated till day 14 after irradiation, and then declined sharply (Fig. 2H). In the same period, TGF-β levels were lower than the normal level at day 14 after irradiation and then recovered to the normal level at day 21 (Fig. 2I). Additionally, we found that the high ratio of IL-1β to TGF-β represented pneumonitis (Fig. 2, E and J).

We questioned whether the high-level of IL-1β induced a subsequent elevation in TGF-β that facilitated chronic pulmonary fibrosis development. So, next we investigated in vitro whether IL-1β could induce TGF-β expression. We found that IL-1β induced both extracellular and intracellular TGF-β production in L929 cells (Fig. 3, A and B). We further demonstrated that under the same concentration IL-1β could directly and more efficiently trigger the expression of TGF-β target gene MMP-9 than could TGF-β (Fig. 3C). To distinguish whether IL-1β-induced TGF-β target gene expression is TGF-β dependent, we employed the SBE reporter gene, because SBE is the key element in the promoter region of TGF-β target genes and the activation of SBE also means the initiation of transcription of TGF-β target genes. Data showed IL-1β directly activated SBE reporter gene within 4 hours in the absence of TGF-β (Fig. 3D), which suggested that IL-1β itself might have activated TGF-β target genes in a TGF-β-independent manner despite the fact that IL-1β could induce TGF-β production in vitro (Fig. 3A and B).

The data above demonstrated that during the development of RILI IL-1β played a central role and its profibrotic effect was mainly owing to its activation of TGF-β target genes.

Naringenin, Like IL-1β Antibody, Protects Against Radiation-Induced Lung Injury by Reducing IL-1β. The abovementioned data demonstrated that IL-1β played the vital role in radiation-induced lung injury. Thus, to study whether

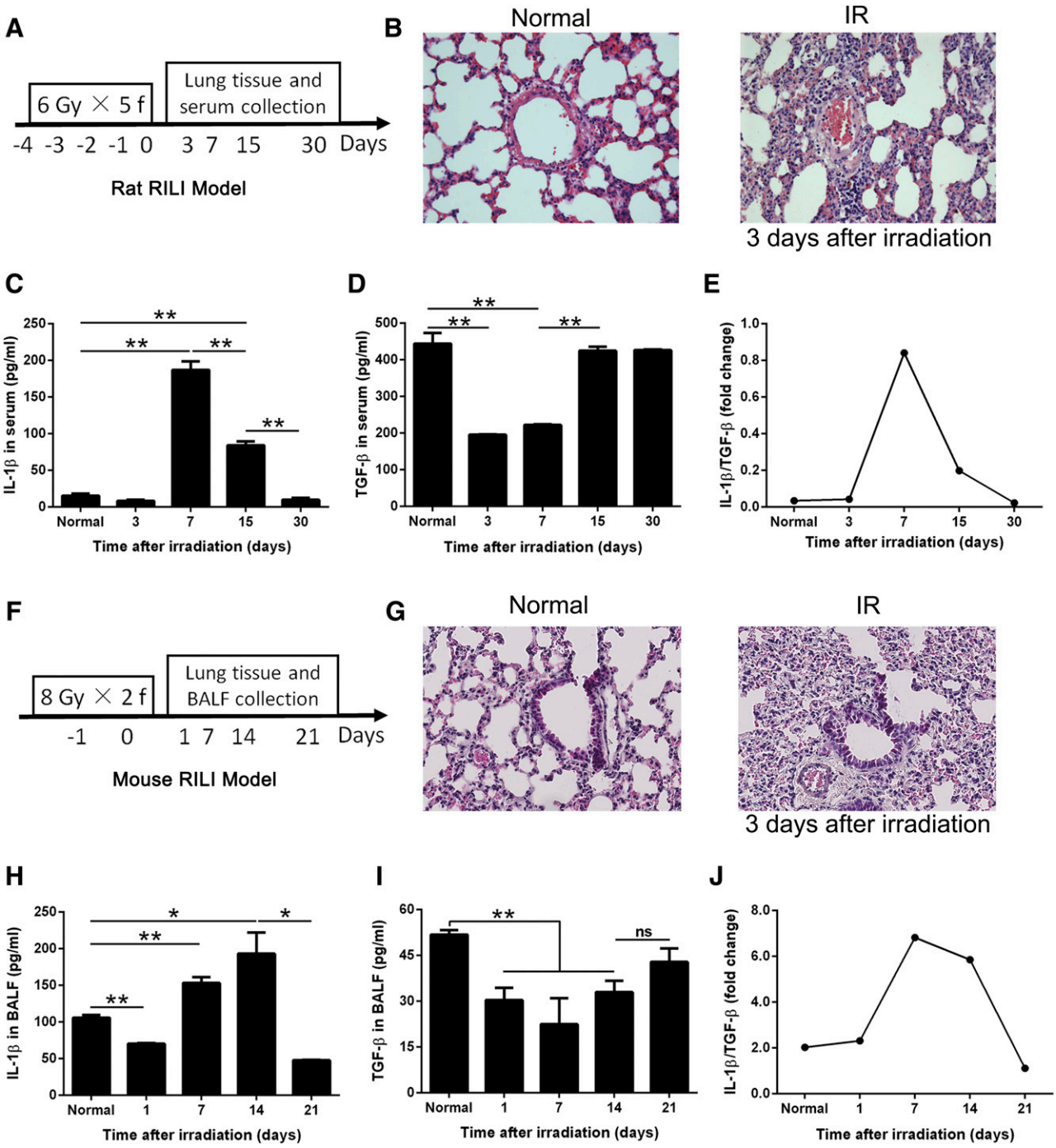

Fig. 2. The changes of IL-1β and TGF-β during radiation-induced lung injury in rodent models. Wister rats (A) were exposed to 6 Gy × 5 F thoracic irradiation (IR) and BALB/c mice (F) were exposed to 8 Gy × 2 F thoracic irradiation (IR). Histologic changes in lungs of rats (B) or mice (G) were assessed by hematoxylin and eosin staining at day 3 after IR (original magnification, 200×). (C) IL-1β and (D) TGF-β in serum of rats at days 3, 7, 15, and 30 after IR were determined by ELISA. (H) IL-1β and (I) TGF-β in BALF of mice at days 1, 7, 14, and 21 after IR were determined by ELISA. Three rats or mice in each group were sacrificed at each timepoint. The ratio of IL-1β over TGF-β was plotted at each time point for rat (E) and mouse (J) models. $^{*}P < 0.05$; $^{**}P < 0.01$. ns, non-significant change.

IL-1β blockade could mitigate the development of RILI, we employed IL-1β neutralizing antibody to block IL-1β in mice receiving thoracic irradiation. IL-1β neutralizing antibody treatment started 1 day before irradiation and continued at a frequency of every 2 days for a total of 10 times (Fig. 4A). On day 3 after irradiation, lung tissues were collected for hematoxylin and eosin staining, and on day 60 after irradiation, lung tissues were collected for Masson staining. Histologic analysis showed radiation-induced interalveolar septa thickening and alveolar space narrowing were ameliorated by IL-1β neutralization at day 3 after irradiation (Fig. 4B). Radiation-induced collagens deposited around the bronchus and vessels were greatly reduced by IL-1β antibody treatment at day 60 after irradiation (Fig. 4, C and D). At day 7 after irradiation, ELISA data showed

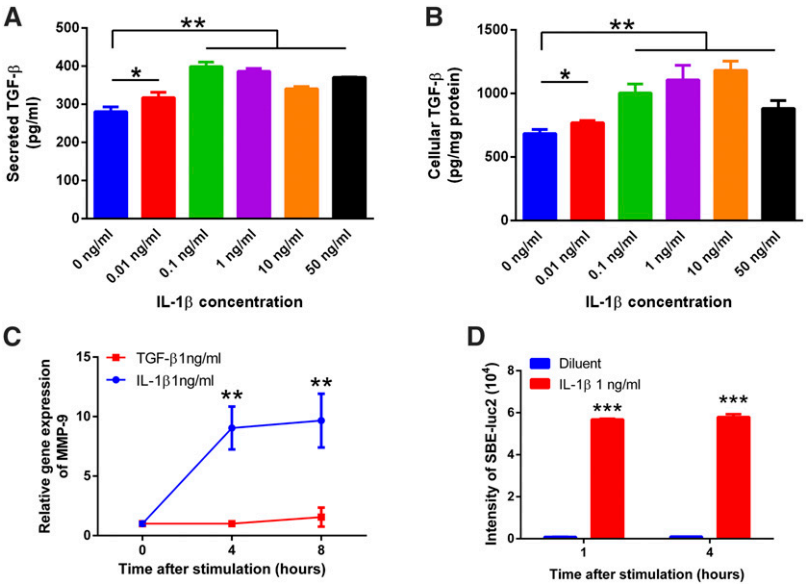

Fig. 3. IL-1β induces TGF-β production and activates TGF-β signaling in vitro. L929 cells were treated with 0, 0.01, 0.1, 1, 10, and 50 ng/ml of IL-1β for 48 hours. TGF-β in supernatant (A) and cell lysate (B) was determined by ELISA. (C) Gene expression of matrix metallopeptidase 9 in L929 was determined at 0, 4, and 8 hours after 1 ng/ml IL-1β or 1 ng/ml TGF-β treatment. (D) The activity of SBE under 1 ng/ml IL-1β treatment of either 1 or 4 hours was detected by luminescence detector. All data are representative of three experimental repeats. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$.

that irradiation resulted in a higher level of IL-1β (24.13 $\pm$ 5.828 pg/ml) in BALF than that of the normal control (8.92 $\pm$ 2.321 pg/ml), whereas the IL-1β in BALF was almost completely neutralized by its antibody (0.931 $\pm$ 0.668 pg/ml) (Fig. 4E).

We next examined whether naringenin exerted its protective effect by reducing radiation-induced IL-1β, for the reason that IL-1β plays an important role in irradiation-induced lung injury. Thus, we employed a combined doses strategy of naringenin treatment to achieve a comprehensive improvement in RILI, as shown in detail in Fig. 4A. Like IL-1β-neutralizing antibody, naringenin markedly deceased the concentration of IL-1β in BALF of mice receiving irradiation (Fig. 4E), and significantly improved RILI in both the early phase of pneumonitis (Fig. 4B) and late phase of lung fibrosis (Fig. 4, C and D).

Our data provided evidence that both IL-1β-targeting strategies, using neutralizing antibody and naringenin, were able to alleviate RILI.

Naringenin Maintains the Homeostasis of Dysregulated Inflammatory Mediators Induced by Irradiation. Upon irradiation a large number of cytokines, chemokines, and growth factors respond and then recruit immune cells to the irradiated field for tissue repair. To investigate whether naringenin's protective effect on RILI involve other factors besides IL-1β, we surveyed changes in 22 inflammatory factors in serum of rats at day 7 after irradiation. Indeed, irradiation caused disorder in these inflammatory factors to varying degrees, among which the elevation of IL-1β was most remarkable. Naringenin treatment brought most of the 22 inflammatory factors to the levels of the normal control group (Fig. 5). Of note, a high level of GRO/KC induced by irradiation indicating more neutrophil infiltration into lung tissue and these neutrophils were potential producers of the high level of IL-1β. Naringenin treatment brought GRO/KC to the basal level of normal controls, suggesting neutrophils would migrate to the lung after irradiation and produce much less IL-1β in this setting. These data demonstrated that by restoring the homeostasis of irradiation-induced disorder of inflammatory factors naringenin exerted a profound radioprotective effect.

Discussion

Inflammasome activation and IL-1β/IL-1R1 signaling were implicated in the development of lung injury caused by chemotherapy, nanoparticles, or infections (Gasse et al., 2007; Palomo et al., 2014; Sun et al., 2015). Indeed, we observed that blockade of IL-1β effectively ameliorated both acute radiation pneumonitis and chronic radiation fibrosis. Consistent with previous reports, the important role of IL-1β in the pathogenesis of RILI suggested that IL-1β is a common mediator in various stimuli-induced lung injury. TGF-β also plays a vital role in the pathogenesis of radiotherapy-induced lung fibrosis, and we provided evidence that TGF-β and its downstream genes could be directly regulated by IL-1β (Lee et al., 2006) (Fig. 3). Moreover, Smad3 knockout mice showed no evidence of fibrosis even if IL-1β was overexpressed (Bonniaud et al., 2005). In liver fibrosis model, IL-1β level was found to be elevated before the onset of hepatic stellate-cell activation and fibrogenesis, which process was dominated by TGF-β signaling (Gieling et al., 2009), but how IL-1β and TGF-β interplayed with each other in RILI remained largely elusive. Our study shows for the first time that during the development of RILI, the ratio of IL-1β to TGF-β after radiation could indicate early radiation pneumonitis. More importantly, IL-1β might directly trigger the TGF-β target genes during RILI to involve later fibrosis.

Up to date, the standard care of radiation pneumonitis remains symptomatic treatment with corticosteroids. Some other agents testing for the prevention of radiation treatment pulmonary fibrosis have been applied in preclinical or clinical studies, such as inhibitors of TNF-α (infliximab), Smads inhibitors (pentoxifylline and relaxin), and antibodies against TGF-β or connective tissue growth factor (FG-3019) (Tsoutsou and Koukourakis, 2006; Tsoutsou, 2014; Raghu et al., 2016). Given the pivotal role of IL-1β in RILI and IL-1β actions upstream of TGF-β, targeting IL-1β could be considered an emerging strategy to treat RILI. In line with our findings, we observed that IL-1β blockade by neutralizing antibody

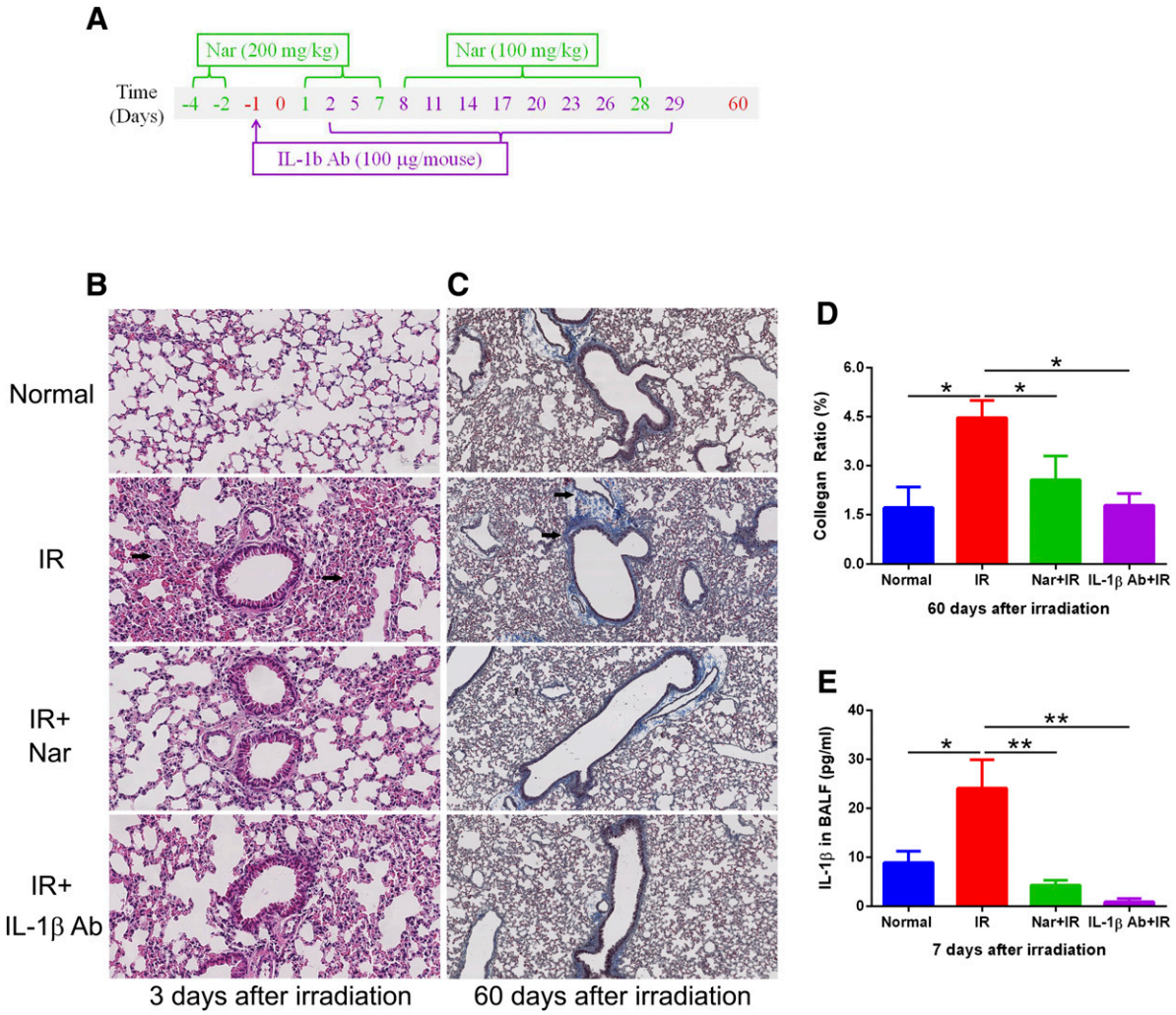

Fig. 4. The protective effect of IL-1β blockade on radiation-induced lung injury. BALB/c mice were exposed to 8 Gy × 2 F thoracic irradiation (IR) and mouse IL-1β antibody (intraperitoneally) or naringenin (oral) was given, as shown schematically in (A). (B) Hematoxylin and eosin staining of lung specimen 3 days after IR (original magnification, 200×) and (C) Masson staining of lung specimen 60 days after IR (original magnification, 70×). (D) The percentage of collagen-positive area in lung was quantified using ImageJ 1.8.0 software on the basis of Masson staining in (C). (E) IL-1β content in BALF at day 7 after IR was detected by ELISA. Three mice in each group were sacrificed at each timepoint. $^{*}P < 0.05$; $^{**}P < 0.01$.

could effectively protect from both radiation-induced early pneumonitis and late fibrosis. Additionally, naringenin exerted protective effect in RILI and also lowered the radiation-induced high level of IL-1β.

Recently, monoclonal neutralizing antibodies and anakinra, a recombinant derivative of IL-1R which antagonize both IL-1β and IL-1α are under clinical development for the treatment of autoimmune diseases (Dinarello, 2011). However clinical data regarding IL-1β blockade in thoracic radiotherapy have been barely reported so far. Other concerns, such as the interference effects of chemotherapy in preclinical studies, may prevent the wide use of IL-1β blockade strategy in cancer therapy as well (Ma et al., 2011; Mattarollo et al., 2011). In tumor bearing mice, we also observed that IL-1β blockade in combination with radiotherapy did not improve the lifespan (Supplemental Fig. 1).

Moreover, during the early stage of RILI, TNF-α and IL-1β are potent proinflammatory cytokines triggering recruitment of inflammatory cells, which release IL-8, IP-10, macrophage inflammatory protein MIP-2 RANTES, MCP-1, eotaxin, MIP-1α, and MIP-1β predominately for chemoattraction of monocytes and lymphocytes, which may be involved in radiation-induced fibrosis (Johnston et al., 1998). Thus, the challenge in the treatment of RILI would be to introduce molecules able to subvert the vicious cascade of cytokine interactions and shift them into anti-fibrotic profile. Therefore, we proposed that a single-cytokine blockade might not comprehensively improve the tumor microenvironment and enhance the antitumor efficacy of radiotherapy. Naringenin treatment protected against radiation-induced early inflammation and late fibrosis in lung as effectively as IL-1β neutralizing antibody. Consistent with our previous findings in a bleomycin-induced lung fibrosis model, naringenin treatment restored the homeostasis of cytokines disordered by radiation, indicating that naringenin's protective effect included but was not limited to anti-inflammation, exemplified by IL-1β repression (Fig. 5). And this advantage of naringenin over anti-IL-1β antibody led to a better antitumor efficacy in tumor-bearing mice receiving radiotherapy (Supplemental Fig. S1). How naringenin regulates IL-1β expression and, further, how naringenin

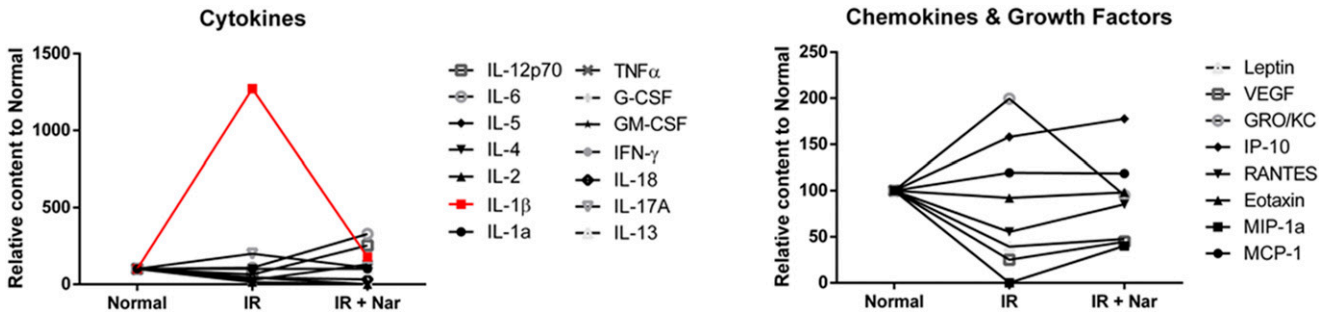

Fig. 5. Naringenin maintains the homeostasis of dysregulated inflammatory mediators induced by irradiation. Wistar rats (n = 3 in each group) were exposed to 6 Gy × 5 f thoracic irradiation (IR), 200 mg/kg of Nar (oral) was administered for 30 days from day 3 before IR. At day 7 after IR, sera were collected for MILLIPLEX assay. The profiles of cytokines, chemokines, and growth factors in normal, IR, and IR + Nar groups are plotted.

maintains the homeostasis of immune responses under irradiation will be the focus of our future research.

In summary, we proved that IL-1β played a vital role in the development of RILI, and blockade of IL-1β significantly ameliorated both acute radiation pneumonitis and chronic radiation fibrosis in rodent models. Like IL-1β antibody, naringenin effectively alleviated RILI. In addition to the reduction of IL-1β, naringngein was also able to maintain the homeostasis of radiation-induced disorder of inflammatory factors. Our investigation suggested that recovering the homeostasis of radiation-induced disorder of inflammatory factors could be a more promising strategy for alleviating radiation-induced lung injury compared with single-cytokine blockade. Accordingly, naringenin may be an effective adjuvant therapeutic agent with thoracic radiotherapy, which warrants further clinical.

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Authorship Contributions

Participated in research design: Chao Zhang, Zeng, Yao, Song, Wei.

Conducted experiments: Chao Zhang, Zeng, Yao, Xu, Wei, Wang, Yin, Barman, F. Zhang.

Performed data analysis: Chao Zhang, Zeng, Yao, Chunling Zhang, F. Zhang, Wei.

Wrote or contributed to the writing of the manuscript: Chao Zhang, Zeng, Yao, Song, Liang.

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