

# Molecular Mechanisms and Treatment of Radiation-Induced Lung Fibrosis

Nian-Hua Ding<sup>1</sup>, Jian Jian Li<sup>2,\*</sup> and Lun-Quan Sun<sup>1,\*</sup>

<sup>1</sup>Center for Molecular Medicine, Xiangya Hospital, Central South University, Changsha, Hunan 410078, China;

<sup>2</sup>Department of Radiation Oncology, NCI-Designated Comprehensive Cancer Center, University of California at Davis, Sacramento, CA 95817, USA

**Abstract:** Radiation-induced lung fibrosis (RILF) is a severe side effect of radiotherapy in lung cancer patients that presents as a progressive pulmonary injury combined with chronic inflammation and exaggerated organ repair. RILF is a major barrier to improving the cure rate and well-being of lung cancer patients because it limits the radiation dose that is required to effectively kill tumor cells and diminishes normal lung function. Although the exact mechanism is unclear, accumulating evidence suggests that various cells, cytokines and regulatory molecules are involved in the tissue reorganization and immune response modulation that occur in RILF. In this review, we will summarize the general symptoms, diagnostics, and current understanding of the cells and molecular factors that are linked to the signaling networks implicated in RILF. Potential approaches for the treatment of RILF will also be discussed. Elucidating the key molecular mediators that initiate and control the extent of RILF in response to therapeutic radiation may reveal additional targets for RILF treatment to significantly improve the efficacy of radiotherapy for lung cancer patients.

**Keywords:** Fibrosis, lung cancer, radiotherapy, side effects.

## 1. INTRODUCTION

Radiation therapy is an essential treatment modality for multiple thoracic malignancies and is a standard treatment for patients with non-small cell lung cancer (NSCLC). However, the efficacy of lung cancer radiotherapy can be severely compromised by the side effects of radiation-induced lung damage, such as pneumonitis and lung fibrosis, which are generally designated as radiation-induced lung fibrosis (RILF) [1]. The most radiosensitive subunit of the lung is the alveolar/capillary complex [2]. Replacement of normal lung parenchyma with fibrotic tissue is a culminating event and is refractory to treatment. Pulmonary fibrosis typically develops between 6 and 24 months post-irradiation and stabilizes after 2 years [3, 4]. There are many factors that influence the time to onset and the severity of radiation-associated pneumonitis, including the volume of the irradiated parenchyma [5, 6], the absorbed radiation dose [2, 7], the number of fractions into which the absorbed dose is divided [8], the size of the individual dose per fraction, the radiation dose rate (the radiotherapy output device), and the adjuvant use of chemotherapy [1, 9, 10]. Patient-related factors, such as pre-existing lung disease [11], poor pulmonary function [11, 12], age [13] and unidentified genetic predispositions [14], also affect the outcome. The clinical data on RILF treatment have not been optimistic. Elucidating the detailed pathology of and obtaining mechanistic insights into RILF is urgently needed for the development of pharmacological and biological therapeutics to treat or mitigate this disease. In this review, we summarize the current understanding of the

factors related to and the potential treatments for RILF and anticipate that novel potential therapeutic approaches will be discovered that significantly reduce and/or eliminate RILF in lung cancer radiotherapy.

## 2. DEFINITION OF RILF

Radiation pneumonitis and RILF are assessed according to the RTOG/EORTC criteria and are classified from grade 0 to grade 5, depending on the clinical manifestations of dry cough, dyspnea and severe respiratory insufficiency [15]. RILF is usually a later complication of lung injury, and it can cause dyspnea, affect the long-term quality of life and result in fatal respiratory insufficiency [16, 17]. RILF is characterized by the accumulation of fibroblasts, myofibroblasts, inflammatory cells and extracellular matrix proteins, such as collagen, with the subsequent formation of a scar, which eventually result in impaired lung function [18, 19]. Because there are numerous contributing factors and various diagnostic settings for RILF, the reported incidence in patients who have received radiotherapy varies significantly, from 1% to 43% [20-25].

## 3. PREDICTING AND DETECTING RILF

### 3.1. Medical Imaging

An early diagnosis of RILF is critical for preventing and treating RILF. Detecting radiation pneumonitis associated with early responses (2-4 weeks post-irradiation) may provide an opportunity to adjust the treatment strategy before the onset of acute pneumonitis and/or irreversible fibrosis. Currently, several methods are utilized to predict and detect RILF in clinical or research settings, including measuring serum factors, screening the genetic background and medical

\*Address correspondence to these authors at the Center for Molecular Medicine, Xiangya Hospital, Central South University, Changsha, Hunan 410078, China; Tel: +86-731-84327646; Fax: +86-731-84327212; Emails: [lunquansun@csu.edu.cn](mailto:lunquansun@csu.edu.cn) and [jian-jian.li@ucdmc.ucdavis.edu](mailto:jian-jian.li@ucdmc.ucdavis.edu)

imaging. Most of the methodologies involving serum factors and genetic information remain in the research stage. In the clinic, medical imaging, which is rapidly growing, has been the preferred method for diagnosing RILF. Although X-rays and CT scans have been widely utilized for lung imaging, it is generally desirable to use a radiation-free imaging system for the lungs, particularly in patients with pulmonary fibrosis. With the recent progress in MRI imaging of the lungs, this tool has become superior to CT in patients who are at risk if they are exposed to extra doses of ionizing radiation. Thind *et al.* demonstrated the feasibility of hyperpolarized  $^{13}\text{C}$  metabolic MR spectroscopy and imaging to detect early RILF [26]. Micro-computed tomography (micro-CT) can detect radiation-induced lung injuries a few months after irradiation and can be utilized for the early detection and assessment of RILF-associated structural and histopathological changes in mice [27].

### 3.2. Factors Detected in Serum, Lung Tissue and Bronchial Lavage(BAL) Fluid

In mice, serum factors, such as colony-stimulating factor (G-CSF), interleukin-6 (IL-6), and keratinocyte-derived chemokines (KCs), have been explored as potential surrogate markers to predict and detect RILF because the serum and tissue levels of these cytokines are positively correlated [28]. The current risk predictors comprise a variety of proinflammatory and profibrotic cytokines and molecules, including TGF $\beta$ 1, that have been implicated in the development and persistence of radiation-induced lung injury [29]. However, individual cytokines and dosimetric parameters are poor independent predictors of RILF, whereas the combination of IL-8 and TGF- $\beta$ 1 levels and the mean lung irradiation dose had an improved predictive ability compared with a single variable [23]. Cytokines can also be detected in BAL fluid and lung tissue lysates. The detectable cytokines in BAL fluid are much less than that in lung tissue lysates or serum. Due to the poor correlation in cytokine levels among lung tissues, serum and BAL, the predictive value of the cytokines in BAL is yet to be defined. The inability to detect other cytokines may be due to the detection limits of the assay in addition to un-optimized assay conditions [28].

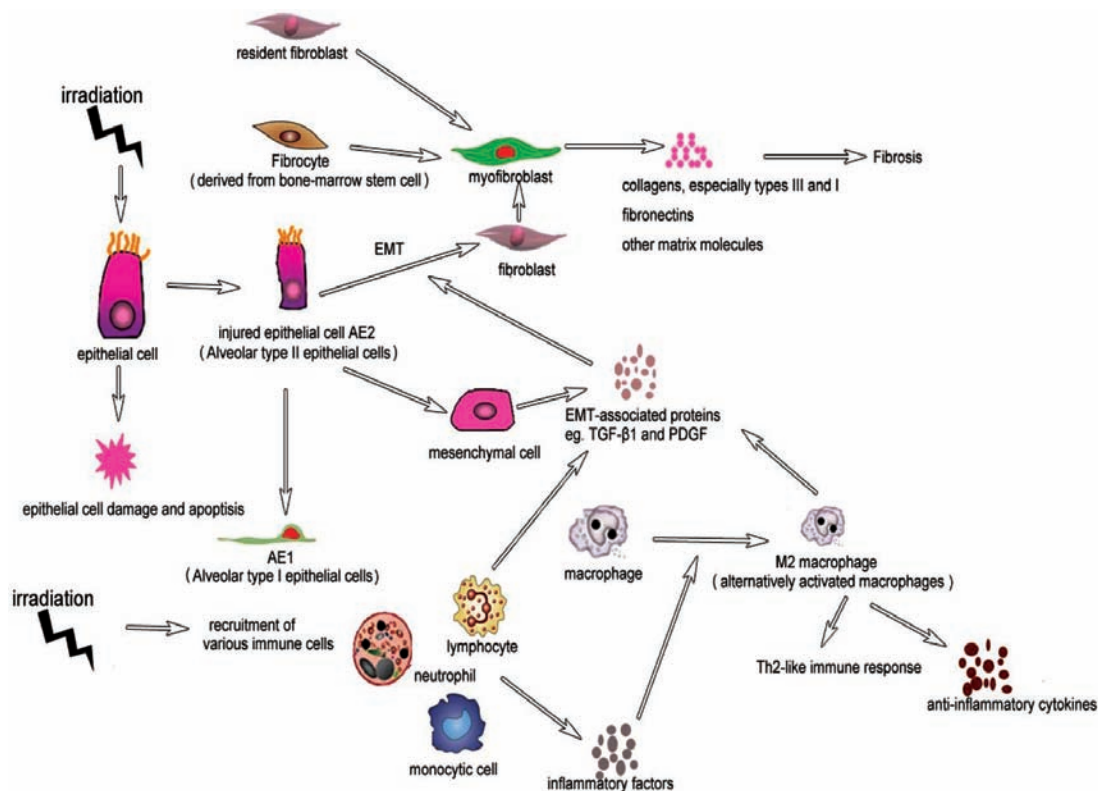
### 3.3. Genomic Markers

The susceptibility to RILF is thought to be associated with genetic background because data has indicated that RILF is a heritable trait in mice. A genome-wide single nucleotide polymorphism (SNP) association study of an inbred mouse strain with prior linkage and gene expression data identified 10 loci that were significantly associated with radiation-induced lung injury; these loci included *Cadm1*, *Slamf6* and *Cdkn1a* [30]. A region of chromosome 17 may harbor a "universal" lung injury gene [31]. Gene expression profiling has been used to distinguish radiation-induced fibrosing alveolitis from alveolitis in mice. To define the gene expression profiles and to identify pathways that influence the alveolitis and fibrosis phenotypes, microarray expression profiling was performed on A/J (late alveolitis response), C3H/HeJ (C3H, early alveolitis response) and C57BL/6J (B6, fibrosis response) mice. The pathway analysis revealed that the expression of complement and B-cell proliferation

and activation genes differed between A/J and C3H mice, thereby distinguishing fibrosis from the alveolitis response and cytokine signaling [32].

## 4. SPECIFIC CELLS INVOLVED IN THE DEVELOPMENT OF RILF

The pathological mechanisms of RILF are complex and involve numerous cell types (Fig. 1). Thorax irradiation causes delayed damage to resident lung cells and typically leads to the apoptosis of primarily bronchiolar epithelial cells and a loss of barrier function. Myofibroblasts, which produce collagens (especially types I and III), fibronectins and other matrix molecules, have been suggested to play a central role in the pathogenesis of pulmonary fibrosis [33-35]. Their origin has become the subject of intense investigation [36]. Although myofibroblasts were believed to derive primarily from resident fibroblasts, recent studies have demonstrated that myofibroblasts can originate from circulating fibroblast-like cells called fibrocytes, which are derived from bone marrow stem cells [37]. Epperly and colleagues demonstrated that marrow-derived cells constituted 20-50% of the cells in fibrotic areas during irradiation-induced fibrosis by transplanting green fluorescent protein-positive bone marrow into wild-type mice [38]. More recent studies have indicated that injured epithelial cells may be a source of myofibroblasts in a process termed epithelial/endothelial-mesenchymal transition (EMT/EndMT) [39-41]. EMT is the process by which fully differentiated epithelial cells undergo a phenotypic transition into migratory mesenchymal cells, often fibroblasts and myofibroblasts [19, 42-44]. It is well established that EMT plays a central role during tumor development and progression. Epiblasts undergo EMT early in development to form the primary mesenchyme. Secondary epithelia are created via mesenchymal-epithelial transitions (MET). These secondary epithelia then differentiate to form fully differentiated adult epithelia or undergo a second round of EMT to form a variety of mesenchymal and connective tissue cells, such as adipocytes, chondrocytes, osteoblasts, myocytes, and fibroblasts [40]. In response to inflammatory stress, more than 30% of the myofibroblasts can arise via EMT, whereas resident fibroblasts contribute only 23% of the myofibroblasts in the kidney [45-47]. Alveolar type II epithelial cells (AE2) from patients with idiopathic pulmonary fibrosis (IPF) expressed high levels of EMT-associated protein markers, suggesting that AE2 cells had acquired a mesenchymal phenotype [48, 49]. Using genetically modified mice in which AE2 cell fate can be tracked, Kim *et al.* found that AE2 cells were the progenitors for mesenchymal cells and contributed significantly to the pool of expanded fibroblasts after lung injury [48]. Tanjore *et al.* reported that approximately one-third of lung fibroblasts were derived from the lung epithelium in a bleomycin-induced lung fibrosis model [50]. After lung injury, increased proliferation/hyperplasia of AE2 cells has frequently been observed [51, 52], which can give rise to alveolar type I epithelial cells to reestablish a functional alveolar epithelium [53]. Recently, it has been proposed that fibroblasts may originate from various sources, such as bone marrow stem cells, resident mesenchymal cells and epithelial cells [33]. Additional studies of each cell population may provide new information on RILF and identify potential therapeutics to reduce the side effects of lung cancer radiotherapy.



**Fig. (1). Numerous cell types are involved in RILF.** Irradiation causes delayed damage to resident lung cells, leading primarily to the injury and apoptosis of bronchiolar epithelial cells. Via EMT, injured epithelial cells provide a source of myofibroblasts, which produce collagens (especially types I and III), fibronectins and other matrix molecules. Myofibroblasts can originate from resident fibroblasts and circulating fibroblast-like cells called fibrocytes, which are derived from bone marrow stem cells. The alveolar type II epithelial cells (AE2) in patients with RILF express high levels of EMT-associated protein markers, suggesting that the AE2 cells had acquired a mesenchymal phenotype. After lung injury, AE2 cells can convert to alveolar type I epithelial cells to re-establish a functional alveolar epithelium. Thorax irradiation can also trigger the recruitment of various immune cells into the lung, such as monocytic cells, neutrophils, basophils and lymphocytes, which are associated with the characteristic changes in the local and systemic expression of cytokines and chemokines.

Macrophages with distinct functions can be divided into two subsets [54-56]: classically activated macrophages (M1 macrophages) that are activated by Toll-like receptor ligands and express pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS), and alternatively activated macrophages (M2 macrophages) that are stimulated by IL-4 or IL-13, secrete anti-inflammatory cytokines, and express arginase 1 (Arg-1). The classical and alternative mechanisms of activating macrophages have been proposed to play a role in radiation-induced pneumonitis and fibrosis, respectively. Studies have demonstrated that iNOS expression increased during the pneumonic stage, whereas Arg-1 expression increased during the fibrotic phase [57]. Macrophage stimulation via IL-4R leads to alternative activation and a positive contribution to the Th2-driven allergic inflammatory response in the lung [58]. M2 macrophages produce growth factors, including TGF- $\beta$ 1 and PDGF, that stimulate epithelial cells and fibroblasts. TGF- $\beta$ 1 is also produced by lymphocytes [59]. Interestingly, irradiation triggers the formation of lipid-loaded macrophages and endothelial cells, which may imply a more general disturbance of lipid metabolism in the irradiated lung [60, 61]. Although the actions of macrophages may not account for the entirety of radiation-induced pulmonary injury, they play an important regulatory role during this process [62].

Thorax irradiation not only affects the macrophages in the lung tissue but also triggers the recruitment of various immune cells into the lung, including monocytic cells, neutrophils, basophils and lymphocytes [60]. The recruitment of immune cells is associated with characteristic changes in local and systemic cytokine and chemokine expression. Additional studies are necessary to define how the various cells interact and are coordinated during irradiation-induced pulmonary injury.

## 5. CYTOKINES AND RELATED FACTORS IN THE DEVELOPMENT OF RILF

### 5.1. TGF- $\beta$

TGF- $\beta$ , which is activated at sites of injury after radiation, plays a critical role in pathological processes. TGF- $\beta$  is produced by numerous inflammatory, mesenchymal and epithelial cells [63, 64] and converts fibroblasts and other cell types into matrix-producing myofibroblasts [34]. TGF- $\beta$  has a multitude of functions, such as controlling the breakdown of connective tissue, inhibiting epithelial cell proliferation, and contributing to the structural changes that occur during airway remodeling in response to asthmatic inflammation by inducing the synthesis of extracellular matrix proteins, such as collagens, and matrix-modifying enzymes,

such as matrix metalloproteinases (MMPs). The principal function of TGF- $\beta$  in the immune system is to inhibit the proliferation and activation of lymphocytes and other leukocytes. Studies have demonstrated that the irradiation of bronchi and alveoli weakly activated TGF- $\beta$ 1 on day 1 and strongly activated it on day 14 post-irradiation. This indicated that particular bronchial and alveolar cells may participate in the complex process of radiation-induced lung fibrosis by acting as cellular sources of active TGF- $\beta$  [65]. It has been suggested that the irradiation-induced activation of TGF- $\beta$ 1 is rapid [66-68]. Prolonged exposure to TGF- $\beta$ 1 (4–6 days) is a powerful stimulus that initiates and maintains EMT in various biological systems and pathophysiological contexts by activating major signaling pathways and transcriptional regulators that are integrated into extensive signaling networks [41, 69, 70].

### 5.2. ECM

Extracellular matrix (ECM) provides physical support to tissues, anchorage sites for cells and a medium for diffusible signaling molecules, such as the enzymes that regulate ECM synthesis and breakdown. ECM is also a major cytokine reservoir and therefore has pleiotropic effects [71]. Zhao *et al.* demonstrated that the expression of vimentin and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) significantly increased after radiation [72], and these molecules are typically used to define the mesenchymal phenotype [73].  $\alpha$ -SMA is released in response to myofibroblast activation and confers strong contractile properties [74]. E-cadherin, present in the plasma membrane of normal epithelial cells, is a calcium-dependent adhesion molecule that plays a significant role in maintaining cellular polarity and morphological structure [75]. The loss of E-cadherin disrupts cell-cell junctions and affects the structural integrity of cells [76]. Snail is a critical transcriptional regulator of E-cadherin that allows epithelial cells to adopt a fibroblast-like phenotype and acquire mesenchymal properties [77]. Collagen-degrading matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are involved in remodeling the extracellular matrix. MMP-2 and MMP-9 are overexpressed during the inflammatory response to radiation-induced lung injury and degrade collagen IV in the basement membrane [78]. MMP13-deficient mice developed less pulmonary fibrosis than their wild-type counterparts [79]. These data indicate that ECM remodeling is necessary in RILF.

### 5.3. NF- $\kappa$ B Network

NF- $\kappa$ B is an active transcription factor in the radiation-induced adaptive response [80]. A cluster of NF- $\kappa$ B-regulated cytokines, including TNF- $\alpha$ , is induced by radiation and contributes to cell sensitivity to radiation [81, 82]. TNF- $\alpha$ , originally described as a cytokine produced by activated T-cells and macrophages, activates NF- $\kappa$ B via receptor activation [83, 84] and regulates the expression of numerous immune and inflammatory response genes [85, 86]. Protein kinase cascades activated by TNF- $\alpha$  phosphorylate and activate I $\kappa$ B kinases and c-jun N-terminal kinase (JNK), both of which are involved in the NF- $\kappa$ B-mediated radiation response [87-89]. Studies of 344 female Fischer rats that were irradiated on the right hemithorax with a fractionated dose of 40 Gy (8 Gy x 5 days) exhibited activated HIF1- $\alpha$  as early as

4 weeks post-irradiation, which paralleled enhanced oxidative stress, tissue hypoxia, macrophage accumulation and NF- $\kappa$ B activity, indicating that the NF- $\kappa$ B and HIF-1 $\alpha$  pathways cooperate during irradiation-induced lung injury and fibrosis [90]. Androgen deprivation by castration significantly augmented RT-induced inflammation involving NF- $\kappa$ B activation and COX-2 expression [91]. Therefore, inhibiting NF- $\kappa$ B signaling networks, especially key cytokines that are upregulated by radiation-induced NF- $\kappa$ B activation, is a potential therapeutic approach for decreasing RILF.

### 5.4. M-CSF and MCP-1

Recent studies have determined that a poorly balanced immune response is a characteristic feature of progressive lung fibrosis in humans. Increased levels of macrophage colony-stimulating factor (M-CSF) and macrophage chemoattractant protein-1 (MCP-1) were observed at days 3 and 21 post-irradiation, respectively [60]. M-CSF is a key regulator of the proliferation, survival and differentiation of monocytes/macrophages, whereas MCP-1 is involved in attracting monocytes, granulocytes and lymphocytes. Both chemokines may promote the influx of immune cells, particularly macrophages, into irradiated lungs. The levels of macrophage inflammatory protein MIP-1 and MIP-2 also increased post-irradiation. Both of these proteins participate in the activation of granulocytes and in the synthesis of proinflammatory cytokines and therefore might foster inflammation-associated lung damage. Cytokines such as MCP-1 and MIPs have therefore been suggested to be profibrotic factors [92].

### 5.5. Inflammatory Factors

The levels of several cytokines involved in the recruitment, proliferation and/or activation of CD4<sup>+</sup> T cells, particularly TH17 cells, increased post-irradiation [60]. During an immune response, naive CD4<sup>+</sup> T helper (Th) cells can differentiate into at least two functional subsets: Th1 cells, which secrete Th1 cytokines, such as INF- $\gamma$ , TNF- $\beta$ , IL-2, IL-12, and Th2 cells, which secrete Th2 cytokines, such as IL-4, IL-5, IL-6, IL-10 and IL-13 [93]. Th1 and Th2 cytokines are crucial to Th1 and Th2 immune reactions, both of which promote the proliferation and differentiation of their respective T cell subsets and inhibit the proliferation and differentiation of the opposing subset [94]. Th2 cytokines have been implicated in asthma, and evidence is accumulating that type 2 immune responses promote the development of pulmonary fibrosis. In mice, Th2 immune response-associated factors (GATA-3, IL-13 and Arg-1) increased after lung irradiation-induced Th2 polarization [95]. Although the exact molecular mechanism leading to fibrosis is unknown, evidence from preclinical models strongly suggests a critical role for Th2-specific signaling in this disease [19, 96].

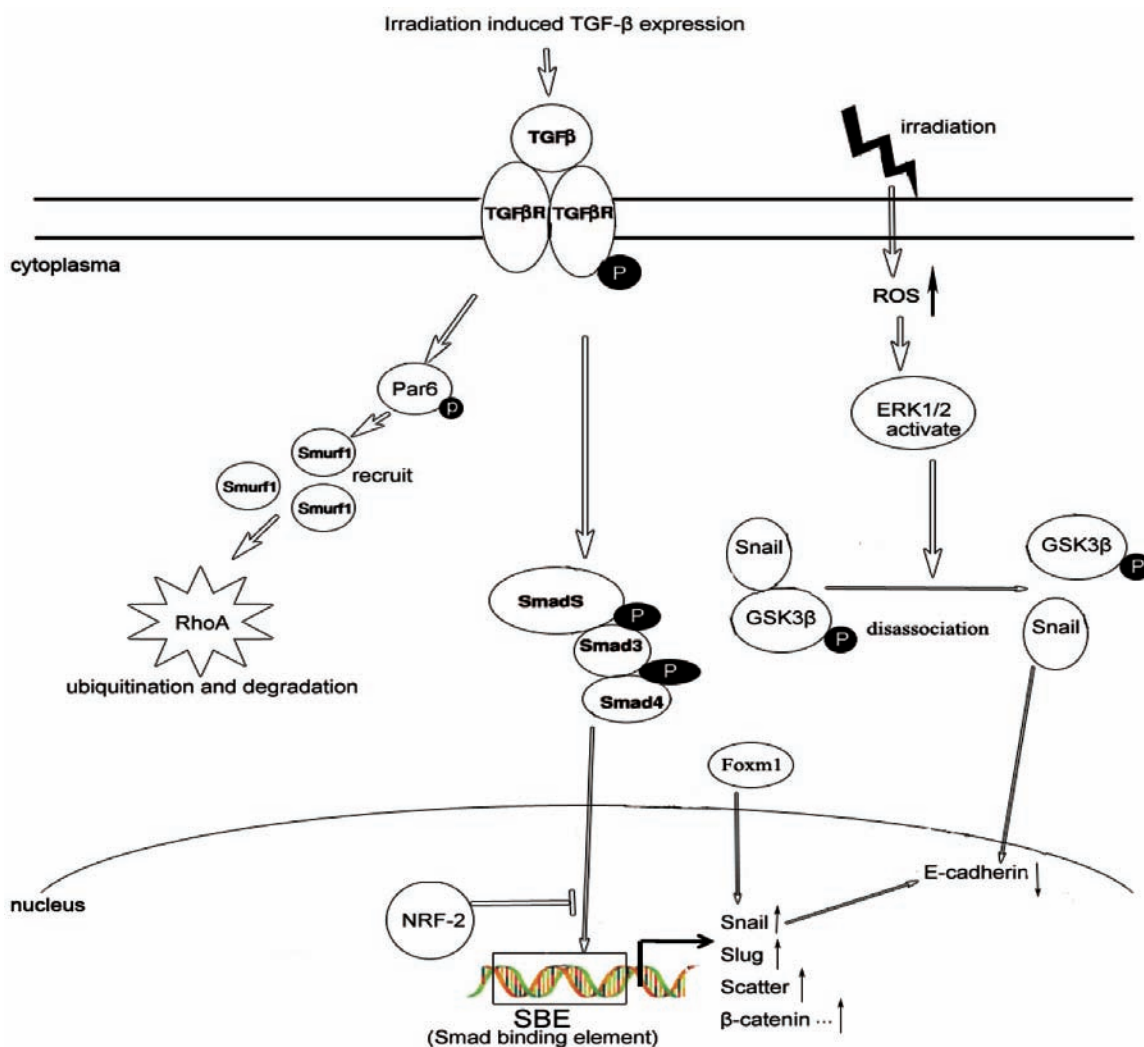
### 5.6. ROS

Radiation interacts with water molecules in biological systems to produce various reactive oxygen species (ROS), including superoxide (O<sup>2-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical ( $\cdot$ OH), which can cause cell damage and cell death [97]. Approximately 60–70% of ionizing radia-

tion-induced cell injury is caused by hydroxyl radicals ( $\cdot\text{OH}$ ) [98, 99]. After irradiation, the increased production of ROS has been linked to parenchymal cell toxicity and to the initiation of a molecular cascade that alters the cytokine milieu in the microenvironment, leading to lipid peroxidation, DNA and protein oxidation, and the activation of proinflammatory factors both *in vitro* and *in vivo* [2, 98, 100, 101]. The lipid peroxidation products stimulate fibrogenic cytokines that act as chemoattractants, mitogens and differentiation inducers for smooth muscle cells in the vessel wall [102]. As such, a complex and multi-level expression network of pro- and anti-inflammatory cytokines, proteases and anti-proteases, and oxidants and antioxidants exists within the lung microenvironment after irradiation. This interactive network governs tissue regeneration, and disrupting this network balance can lead to pulmonary injury.

## 6. ADDITIONAL SIGNALING PATHWAYS INVOLVED IN RILF

Phenotypic changes in various cell populations in the lung and alterations in gene expression triggered by radiation are controlled by multiple regulatory mechanisms and signaling pathways (Fig. 2). Among them, TGF- $\beta$ /Smad signaling is critical and promotes many facets of pulmonary fibrosis, such as ROS generation, myofibroblast and fibrocyte activation and the synthesis of extracellular matrix components. In epithelial cells, the stimulation of TGF- $\beta$  leads to the induction of Smad proteins, which are transcription factors that also activate other transcription factors, including Slug, Snail, Scatter, lymphoid enhancing factor-1, and  $\beta$ -catenin [103]. Recently, FoxM1 was found to bind to and increase the activity of the Snail1 gene promoter [104]. These tran-



**Fig. (2). Signaling pathways in RILF.** Irradiation induces TGF- $\beta$  expression. Ligand binding to TGF- $\beta$  activates type II TGF- $\beta$  receptor serine/threonine kinases. TGF- $\beta$  stimulation results in the induction of Smad proteins, which are transcription factors that also induce other transcription factors, including Slug, Snail, Scatter, lymphoid enhancing factor-1, and  $\beta$ -catenin. FoxM1 binds to and increases the promoter activity of the Snail1 gene. NRF2 binding to a Smad Binding Element (SBE) suppresses TGF- $\beta$  target gene expression. TGF- $\beta$ 1 also activates non-Smad-mediated signaling pathways, which are associated with occludin at tight junctions, to phosphorylate Par6. Phosphorylated Par6 recruits Smurf1, resulting in the ubiquitination and degradation of RhoA, which is responsible for stress fiber formation and the maintenance of apical-basal polarity and junctional stability. Radiation activates the MEK/ERK signaling pathway by increasing ROS generation. Activated ERK1/2 phosphorylates GSK3 $\beta$ , resulting in its inactivation and leading to the disassociation of GSK3 $\beta$  and Snail. Unbound Snail then migrates to the nucleus and represses E-cadherin, leading to a mesenchymal-like phenotypic change.

scription factors result in the expression of the “EMT proteome”, which includes the cellular machinery necessary for the functional disassembly and rearrangement of the cytoskeleton and for cellular motility [41]. The majority of Smad-dependent target genes are controlled by Smad3 [105], which partners with Smad4 upon activation by TGF- $\beta$  receptor serine/threonine kinases and translocates to the nucleus where Smad complexes control target gene transcription by interacting with specific binding motifs in the gene regulatory regions [106]. In Smad3-deficient mice, irradiation-induced dermal fibrosis and renal interstitial fibrosis induced by unilateral ureteral obstruction were both reduced, suggesting an important role for Smads in the TGF- $\beta$  signaling pathway [33, 107]. The transcription factor NRF2 controls antioxidant gene expression, regulates the cellular oxidant burden and represses TGF- $\beta$ /Smad-mediated signaling. NRF2 binding to a Smad Binding Element (SBE) in the proximal promoter of the target gene PAI-1 (a TGF- $\beta$ /Smad-responsive profibrotic gene) suppresses TGF- $\beta$  target gene expression [108, 109].

TGF- $\beta$ 1 also activates non-Smad-mediated cellular signaling pathways, the most important of which involves Rho kinase, which directly activates the cellular machinery necessary for cytoskeletal rearrangement, basement membrane detachment, and E-cadherin down-regulation [110]. Ligand binding to TGF- $\beta$  enables type II TGF- $\beta$  receptor kinase, which is associated with occludin at tight junctions, to phosphorylate Par6 [111]. Par6 is a key component of epithelial polarity complexes that regulate the assembly of tight junctions [112]. This direct protein-protein interaction is independent of Smad proteins. Par6 phosphorylation allows for the recruitment of Smurf1, leading to the ubiquitination and degradation of RhoA, a small GTPase responsible for stress fiber formation and the maintenance of apical-basal polarity and junctional stability [113]. Recently, the modulation of the Rho/ROCK and Smad pathways using Y-27632 (a Rho/ROCK inhibitor) or statins was found to decrease or delay cardiac and pulmonary radiation injury and bleomycin-induced pulmonary fibrosis [114].

Cross-talk between classical TGF- $\beta$  pathways and signaling molecules such as Rho, Ras, extracellular signal-related kinase (ERK), p38 mitogen-activated protein kinase (MAPK), Notch, Wnts, nuclear factor- $\kappa$ B, and PI3K has been demonstrated to affect the extent and reversibility of EMT [41]. Radiation activates the MEK/ERK signaling pathway by increasing ROS generation. Activated ERK1/2 phosphorylates and inactivates GSK3 $\beta$ , leading to the disassociation of GSK3 $\beta$  and Snail. Unbound Snail then migrates to the nucleus and represses E-cadherin, eventually leading to a mesenchymal-like phenotypic alteration of RLE-6TN cells [72]. NF- $\kappa$ B is predominantly activated by exposure to ionizing radiation and plays an important role in the radiation-induced cellular response [80]. Exposure to radiation for more than 6 months causes a sustained elevation in the DNA binding activity of NF- $\kappa$ B. The NF- $\kappa$ B DNA binding complexes switch from p50-p65 heterodimers in normal lung tissue to p50 homodimers in irradiated lung tissue [115]. Therefore, the unique signal pathways and associated molecules may provide opportunities for targeted intervention in radiation-induced lung injury.

## 7. POTENTIAL TARGETS AND TREATMENTS FOR RILF

The significant clinical need for RILF treatments has prompted intense research and development activities both in pharmaceutical companies and academic institutions [25]. The current approaches to the disease have focused on several aspects of the current understanding of the molecular pathology of RILF (Table 1).

### 7.1. Cytokines and Growth Factors

The activation of the TGF- $\beta$ /Smad signal transduction pathway is a critical step in the development of fibrosis and has therefore been explored as a potential intervention point. A small molecule inhibitor of TGF- $\beta$ RI, SM16, reduced the extent of radiation-induced lung injury in a rat model [116]. LY2109761 (a small molecule inhibitor of TGF- $\beta$  receptor I serine/threonine kinase), which reduces p-Smad2 and p-Smad1 expression, suppressed the expression of genes involved in canonical and noncanonical TGF- $\beta$  signaling and reduced inflammation and pulmonary fibrosis, resulting in prolonged survival [117]. Neutralizing antibodies against TGF- $\beta$  inhibited both the radiation-induced reduction in clonogenicity of rat lung fibroblasts and the radiation-induced terminal differentiation of progenitor fibroblasts (MF) to post-mitotic fibrocytes (PMF) [118]. SB203580 and WP631 inhibited the Smad signal transduction pathway, abrogated the excessive proliferation and reduced the expression of p21 (WAF1/CIP1) and PAI-1 (a TGF- $\beta$ /Smad-responsive profibrotic gene [108]) induced by gamma rays and TGF- $\beta$ 1 [119]. MyD88 (a key intracellular adaptor for TLR signaling) regulates innate immunity and NF- $\kappa$ B-activated responses, attenuates long-term radiation-induced lung injury, and protects against fibrosis by mitigating chronic lung injury [120]. We determined that fluorofenidone (1-(3-fluorophenyl)-5-methyl-2-(1H)-pyridone, AKF-PD), a novel pyridone antifibrotic agent, reduced cardiac and kidney fibrosis by inhibiting CTGF (connective tissue growth factor) expression [121, 122].

### 7.2. ROS

The presence of hypoxia in lung tissue after either single or fractionated irradiation is associated with the continuous macrophage-associated production of reactive oxygen/nitrogen species (ROS/RNS), leading to pulmonary tissue damage and fibrosis. Targeting chronic ROS/RNS production after radiation (RT) exposure with the long-term administration of catalytic manganese (Mn) porphyrin-based superoxide dismutase (SOD) mimetics reduces oxidative stress, tissue hypoxia, cytokine production, and lung injury. The recombinant protein SOD-TAT protects against radiation-induced lung injury in mice [123]. Molecular hydrogen (H<sub>2</sub>) is an efficient antioxidant that diffuses rapidly across the cell membrane, reduces ROS levels and suppresses oxidative stress-induced injury in several organs [99]. The active metabolite of amifostine is a radical scavenger, and this drug has potential as a therapeutic for the treatment of radiation-induced lung damage [124].

### 7.3. Targeting Cell Death

Numerous studies have documented the role of TNF- $\alpha$  in fibrotic disease [125, 126]. It is generally accepted that

**Table 1. Current Status of Molecular Interventions for RILF**

Name	Molecular Type	Target/Action	Stage	Reference
SM16	Antibody	Anti-TGF- $\beta$ type 1 receptor	Animal testing	[116]
LY2109761	Quinoline-derived compound	Dual inhibitor of TGF- $\beta$ receptor types I and II	Animal testing	[117]
AKF-PD	Pyridone	Inhibitor of connective tissue growth factor expression	Animal testing	[121, 122]
TGF- $\beta$ inhibitor	Antibody	Anti-TGF- $\beta$	Animal testing	[118]
SB203580	Pyridinylimidazole compound	Inhibitor of TGF- $\beta$ /Smad signal transduction	Cellular assays	[119]
WP631	Bisintercalating anthracycline antibiotic	DNA intercalator, inhibits cell proliferation	Cellular assays	[119]
MyD88	Recombinant protein	A key intracellular adaptor of TLR signaling, regulates innate immunity	Animal testing	[120]
SOD-TAT	Recombinant protein	Targets oxidative damage	Animal testing	[123]
Amifostine	Phosphate compound	Scavenges oxygen free radicals to reduce oxidative damage	In clinical use	[124]
H <sub>2</sub>	Hydrogen molecule	Antioxidant that reduces ROS and suppresses oxidative stress-induced injury	In cell and animal testing	[99]
TNF- $\alpha$ receptor I expression vector	Plasmid	Inhibitor of TNF- $\alpha$ activity	In cell and animal testing	[125]
Glucocorticosteroids	Steroid	Immunoregulation, restoration of the immunological balance	In clinical use	[130]
Sivelestat	Small molecule	Inhibitor of neutrophil elastase, decreases collagen deposition and the accumulation of neutrophils	In clinical use	[131]

TNF- $\alpha$  is a key mediator in the pathogenesis of post-radiation lung injury and causes cachexia, tissue damage and irreversible shock effects. Therefore, fibrotic injury could be prevented with anti-TNF antibodies. Recently, there has been a growing interest in the potential of the TNF- $\alpha$  soluble receptors I and II as inhibitors of normal tissue injury caused by radiation or chemotherapy [127]. The antifibrotic activity after lung injury of gene constructs that encode soluble c-II and TNFR-I was tested *in vivo* by local gene delivery [128, 129].

#### 7.4. Targeting Inflammatory Cells

It has been suggested that restoring the immunological balance represents an important therapeutic intervention strategy to treat post-radiation lung injuries. Glucocorticosteroids are beneficial in many irradiated organs and tissues [130]. The neutrophil elastase inhibitor Sivelestat significantly decreased collagen deposition and neutrophil accumulation in the lung parenchyma and improved the static lung compliance of injured lungs [131].

#### CONCLUSIONS AND PERSPECTIVES

In summary, there are numerous cells, mediators and signaling pathways that are involved in the initiation and progression of RILF, suggesting multiple potential complex mechanisms for the prevention and treatment of this disease. Novel sensitive and reliable biomarkers for the diagnosis of

RILF in cancer patients have yet to be validated, especially those that predict the early phase of pulmonary injury after different radiotherapy regimens [132]. Elucidating the mechanisms of radiation-induced inflammatory responses may enable the identification of sensitive circulating plasma markers that are easy to detect and effective targets for the prevention and/or treatment of RILF in lung cancer patients.

#### CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

#### ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (Grant No. 2013CB967203) and by the US National Institutes of Health (Grant RO1 CA152313-01-A1; J.J.L.).

#### REFERENCES

- [1] Mehta V. Radiation pneumonitis and pulmonary fibrosis in non-small-cell lung cancer: pulmonary function, prediction, and prevention. *Int J Radiat Oncol Biol Phys* 2005; 63: 5-24.
- [2] Ghafoori P, Marks LB, Vujaskovic Z, Kelsey CR. Radiation-induced lung injury. Assessment, management, and prevention. *Oncology (Williston Park)* 2008; 22: 37-47; discussion 52-3.
- [3] McDonald S, Rubin P, Phillips TL, Marks LB. Injury to the lung from cancer therapy: clinical syndromes, measurable endpoints,

- and potential scoring systems. *Int J Radiat Oncol Biol Phys* 1995; 31: 1187-203.
- [4] Medhora M, Gao F, Jacobs ER, Moulder JE. Radiation damage to the lung: mitigation by angiotensin-converting enzyme (ACE) inhibitors. *Respirology* 2012; 17: 66-71.
- [5] Fan M, Marks LB, Lind P, *et al.* Relating radiation-induced regional lung injury to changes in pulmonary function tests. *Int J Radiat Oncol Biol Phys* 2001; 51:311-7.
- [6] Fu XL, Huang H, Bentel G, *et al.* Predicting the risk of symptomatic radiation-induced lung injury using both the physical and biologic parameters V(30) and transforming growth factor beta. *Int J Radiat Oncol Biol Phys* 2001; 50:899-908.
- [7] Libshitz HI. Radiation changes in the lung. *Semin Roentgenol* 1993; 28:303-20.
- [8] Roach M, 3rd, Gandara DR, Yuo HS, *et al.* Radiation pneumonitis following combined modality therapy for lung cancer: analysis of prognostic factors. *J Clin Oncol* 1995; 13: 2606-12.
- [9] Lee JS, Scott C, Komaki R, *et al.* Concurrent chemoradiation therapy with oral etoposide and cisplatin for locally advanced inoperable non-small-cell lung cancer: radiation therapy oncology group protocol 91-06. *J Clin Oncol* 1996; 14: 1055-64.
- [10] Taghian AG, Assaad SI, Niemierko A, *et al.* Risk of pneumonitis in breast cancer patients treated with radiation therapy and combination chemotherapy with paclitaxel. *J Natl Cancer Inst* 2001; 93: 1806-11.
- [11] Rancati T, Ceresoli GL, Gagliardi G, Schipani S, Cattaneo GM. Factors predicting radiation pneumonitis in lung cancer patients: a retrospective study. *Radiother Oncol* 2003; 67: 275-83.
- [12] Inoue A, Kunitoh H, Sekine I, Sumi M, Tokuyue K, Saijo N. Radiation pneumonitis in lung cancer patients: a retrospective study of risk factors and the long-term prognosis. *Int J Radiat Oncol Biol Phys* 2001; 49: 649-55.
- [13] Kharofa J, Gore E. Symptomatic radiation pneumonitis in elderly patients receiving thoracic irradiation. *Clin Lung Cancer* 2013; 14: 283-7.
- [14] Tsoutsou PG, Koukourakis MI. Radiation pneumonitis and fibrosis: mechanisms underlying its pathogenesis and implications for future research. *Int J Radiat Oncol Biol Phys* 2006; 66: 1281-93.
- [15] Vasic L, Durdevic P. [Radiation-induced lung damage--etiopathogenesis, clinical features, imaging findings and treatment]. *Med Pregl* 2012; 65: 319-25.
- [16] Oh YT, Noh OK, Jang H, *et al.* The features of radiation induced lung fibrosis related with dosimetric parameters. *Radiother Oncol* 2012; 102: 343-6.
- [17] Mazon R, Etienne-Mastroianni B, Perol D, *et al.* Predictive factors of late radiation fibrosis: a prospective study in non-small cell lung cancer. *Int J Radiat Oncol Biol Phys* 2010; 77: 38-43.
- [18] Sime PJ, O'Reilly KM. Fibrosis of the lung and other tissues: new concepts in pathogenesis and treatment. *Clin Immunol* 2001; 99: 308-19.
- [19] Sime PJ. The antifibrogenic potential of PPARgamma ligands in pulmonary fibrosis. *J Investig Med* 2008; 56: 534-8.
- [20] Kong FM, Ten Haken R, Eisbruch A, Lawrence TS. Non-small cell lung cancer therapy-related pulmonary toxicity: an update on radiation pneumonitis and fibrosis. *Semin Oncol* 2005; 32: S42-54.
- [21] Movsas B, Raffin TA, Epstein AH, Link CJ, Jr. Pulmonary radiation injury. *Chest* 1997; 111: 1061-76.
- [22] Arpin D, Mahe MA, Servois V, Claude L. [Predictive factors for acute radiation pneumonitis]. *Rev Pneumol Clin* 2009; 65: 177-86.
- [23] Stenmark MH, Cai XW, Shedden K, *et al.* Combining physical and biologic parameters to predict radiation-induced lung toxicity in patients with non-small-cell lung cancer treated with definitive radiation therapy. *Int J Radiat Oncol Biol Phys* 2012; 84: e217-22.
- [24] Reinfuss M, Mucha-Malecka A, Walasek T, *et al.* Palliative thoracic radiotherapy in non-small cell lung cancer. An analysis of 1250 patients. Palliation of symptoms, tolerance and toxicity. *Lung Cancer* 2011; 71: 344-9.
- [25] Williams JP, Johnston CJ, Finkelstein JN. Treatment for radiation-induced pulmonary late effects: spoiled for choice or looking in the wrong direction? *Curr Drug Targets* 2010; 11: 1386-94.
- [26] Thind K, Chen A, Friesen-Waldner L, *et al.* Detection of radiation-induced lung injury using hyperpolarized (<sup>13</sup>C) magnetic resonance spectroscopy and imaging. *Magn Reson Med* 2012.
- [27] Saito S, Murase K. Detection and early phase assessment of radiation-induced lung injury in mice using micro-CT. *PLoS One* 2012; 7: e45960.
- [28] Ao X, Zhao L, Davis MA, Lubman DM, Lawrence TS, Kong FM. Radiation produces differential changes in cytokine profiles in radiation lung fibrosis sensitive and resistant mice. *J Hematol Oncol* 2009; 2: 6.
- [29] Madani I, De Ruyck K, Goeminne H, De Neve W, Thierens H, Van Meerbeek J. Predicting risk of radiation-induced lung injury. *J Thorac Oncol* 2007; 2: 864-74.
- [30] Paun A, Haston CK. Genomic and genome-wide association of susceptibility to radiation-induced fibrotic lung disease in mice. *Radiother Oncol* 2012; 105: 350-7.
- [31] Haston CK, Zhou X, Gumbiner-Russo L, *et al.* Universal and radiation-specific loci influence murine susceptibility to radiation-induced pulmonary fibrosis. *Cancer Res* 2002; 62: 3782-8.
- [32] Paun A, Lemay AM, Haston CK. Gene expression profiling distinguishes radiation-induced fibrosing alveolitis from alveolitis in mice. *Radiat Res* 2010; 173: 512-21.
- [33] Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008; 214: 199-210.
- [34] Yarnold J, Brotons MC. Pathogenetic mechanisms in radiation fibrosis. *Radiother Oncol* 2010; 97: 149-61.
- [35] Grgic I, Duffield JS, Humphreys BD. The origin of interstitial myofibroblasts in chronic kidney disease. *Pediatr Nephrol* 2012; 27: 183-93.
- [36] Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994; 1: 71-81.
- [37] Quan TE, Cowper SE, Bucala R. The role of circulating fibrocytes in fibrosis. *Curr Rheumatol Rep* 2006; 8: 145-50.
- [38] Epperly MW, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2003; 29: 213-24.
- [39] Zeisberg EM, Tarnavski O, Zeisberg M, *et al.* Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007; 13: 952-61.
- [40] Willis BC, duBois RM, Borok Z. Epithelial origin of myofibroblasts during fibrosis in the lung. *Proc Am Thorac Soc* 2006; 3: 377-82.
- [41] Zavadil J, Bottinger EP. TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene* 2005; 24: 5764-74.
- [42] Savagner P. The epithelial-mesenchymal transition (EMT) phenomenon. *Ann Oncol* 2010; 21 Suppl 7: vii89-92.
- [43] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* 2011; 331: 1559-64.
- [44] Leopold PL, Vincent J, Wang H. A comparison of epithelial-to-mesenchymal transition and re-epithelialization. *Semin Cancer Biol* 2012; 22: 471-83.
- [45] Zeisberg M, Yang C, Martino M, *et al.* Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. *J Biol Chem* 2007; 282: 23337-47.
- [46] Saika S, Kono-Saika S, Ohnishi Y, Sato M, Muragaki Y, Ooshima A, *et al.* Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. *Am J Pathol* 2004; 164: 651-63.
- [47] Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; 119: 1420-8.
- [48] Kim KK, Kugler MC, Wolters PJ, *et al.* Alveolar epithelial cell mesenchymal transition develops *in vivo* during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci U S A* 2006; 103: 13180-5.
- [49] Marmai C, Sutherland RE, Kim KK, *et al.* Alveolar epithelial cells express mesenchymal proteins in patients with idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2011; 301: L71-8.
- [50] Tanjore H, Xu XC, Polosukhin VV, *et al.* Contribution of epithelial-derived fibroblasts to bleomycin-induced lung fibrosis. *Am J Respir Crit Care Med* 2009; 180: 657-65.
- [51] Coggle JE. Proliferation of type II pneumocytes after X-irradiation. *Int J Radiat Biol Relat Stud Phys Chem Med* 1987; 51: 393-9.
- [52] McCormack FX, King TE, Jr., Bucher BL, Nielsen L, Mason RJ. Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1995; 152: 751-9.
- [53] Adamson IY, Young L, Bowden DH. Relationship of alveolar epithelial injury and repair to the induction of pulmonary fibrosis. *Am J Pathol* 1988; 130: 377-83.



- [54] Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *J Clin Invest* 2012; 122: 787-95.
- [55] Varin A, Gordon S. Alternative activation of macrophages: immune function and cellular biology. *Immunobiology* 2009; 214: 630-41.
- [56] Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity* 2010; 32: 593-604.
- [57] Zhang H, Han G, Liu H, *et al.* The development of classically and alternatively activated macrophages has different effects on the varied stages of radiation-induced pulmonary injury in mice. *J Radiat Res* 2011; 52: 717-26.
- [58] Ford AQ, Dasgupta P, Mikhailenko I, Smith EM, Noben-Trauth N, Keegan AD. Adoptive transfer of IL-4Ralpha+ macrophages is sufficient to enhance eosinophilic inflammation in a mouse model of allergic lung inflammation. *BMC Immunol* 2012; 13: 6.
- [59] Assouan RK, Fleurdeley BE, Stevenson HC, *et al.* Expression and secretion of type beta transforming growth factor by activated human macrophages. *Proc Natl Acad Sci USA* 1987; 84: 6020-4.
- [60] Cappuccini F, Eldh T, Bruder D, *et al.* New insights into the molecular pathology of radiation-induced pneumopathy. *Radiation Oncol* 2011; 101: 86-92.
- [61] Chiang CS, Liu WC, Jung SM, *et al.* Compartmental responses after thoracic irradiation of mice: strain differences. *Int J Radiat Oncol Biol Phys* 2005; 62: 862-71.
- [62] Park HR, Jo SK, Yu DK, Jung U. Fractionated irradiations lead to chronic allergic airway inflammation through increasing the influx of macrophages. *Inflamm Res* 2013; 62: 27-36.
- [63] Thompson HG, Mih JD, Krasieva TB, Tromberg BJ, George SC. Epithelial-derived TGF-beta2 modulates basal and wound-healing subepithelial matrix homeostasis. *Am J Physiol Lung Cell Mol Physiol* 2006; 291: L1277-85.
- [64] Khalil N, Bereznyay O, Sporn M, Greenberg AH. Macrophage production of transforming growth factor beta and fibroblast collagen synthesis in chronic pulmonary inflammation. *J Exp Med* 1989; 170: 727-37.
- [65] Mattos MD, Kimura ET, Silva MR, Egami MI, Segreto RA, Segreto HR. [Latent TGFbeta1 activation in the lung irradiated *in vivo*]. *Rev Assoc Med Bras* 2002; 48: 329-34.
- [66] Biswas S, Guix M, Rinehart C, *et al.* Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest* 2007; 117: 1305-13.
- [67] Barcellos-Hoff MH, Derynck R, Tsang ML, Weatherbee JA. Transforming growth factor-beta activation in irradiated murine mammary gland. *J Clin Invest* 1994; 93: 892-9.
- [68] Travis EL, Rachakonda G, Zhou X, *et al.* NRF2 deficiency reduces life span of mice administered thoracic irradiation. *Free Radic Biol Med* 2011; 51: 1175-83.
- [69] Janda E, Lehmann K, Killisch I, *et al.* Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J Cell Biol* 2002; 156: 299-313.
- [70] Bhowmick NA, Ghiassi M, Bakin A, *et al.* Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol Biol Cell* 2001; 12: 27-36.
- [71] Barcellos-Hoff MH. How do tissues respond to damage at the cellular level? The role of cytokines in irradiated tissues. *Radiat Res* 1998; 150: S109-20.
- [72] Nagarajan D, Melo T, Deng Z, Almeida C, Zhao W. ERK/GSK3beta/Snail signaling mediates radiation-induced alveolar epithelial-to-mesenchymal transition. *Free Radic Biol Med* 2012; 52: 983-92.
- [73] Willis BC, Liebler JM, Luby-Phelps K, *et al.* Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol* 2005; 166: 1321-32.
- [74] Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol* 2007; 170: 1807-16.
- [75] Saha B, Chaiwun B, Imam SS, *et al.* Overexpression of E-cadherin protein in metastatic breast cancer cells in bone. *Anticancer Res* 2007; 27: 3903-8.
- [76] Mohamet L, Hawkins K, Ward CM. Loss of function of e-cadherin in embryonic stem cells and the relevance to models of tumorigenesis. *J Oncol* 2011; 2011: 352616.
- [77] Yang Z, Rayala S, Nguyen D, Vadlamudi RK, Chen S, Kumar R. Pak1 phosphorylation of snail, a master regulator of epithelial-to-mesenchyme transition, modulates snail's subcellular localization and functions. *Cancer Res* 2005; 65: 3179-84.
- [78] Yang K, Palm J, Konig J, *et al.* Matrix-Metallo-Proteinases and their tissue inhibitors in radiation-induced lung injury. *Int J Radiat Biol* 2007; 83: 665-76.
- [79] Flechsig P, Hartenstein B, Teurich S, *et al.* Loss of matrix metalloproteinase-13 attenuates murine radiation-induced pulmonary fibrosis. *Int J Radiat Oncol Biol Phys* 2010; 77: 582-90.
- [80] Ahmed KM, Li JJ. NF-kappa B-mediated adaptive resistance to ionizing radiation. *Free Radic Biol Med* 2008; 44: 1-13.
- [81] Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation. *Proc Natl Acad Sci USA* 1989; 86: 10104-7.
- [82] Chen M, Quintans J, Fuks Z, Thompson C, Kufe DW, Weichselbaum RR. Suppression of Bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor alpha, and ceramide. *Cancer Res* 1995; 55: 991-4.
- [83] Blonska M, You Y, Geleziunas R, Lin X. Restoration of NF-kappaB activation by tumor necrosis factor alpha receptor complex-targeted MEKK3 in receptor-interacting protein-deficient cells. *Mol Cell Biol* 2004; 24: 10757-65.
- [84] Osborn L, Kunkel S, Nabel GJ. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor kappa B. *Proc Natl Acad Sci USA* 1989; 86: 2336-40.
- [85] Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol* 1994; 12: 141-79.
- [86] Baeuerle PA, Baltimore D. NF-kappa B: ten years after. *Cell* 1996; 87: 13-20.
- [87] Bradbury CM, Markovina S, Wei SJ, *et al.* Indomethacin-induced radiosensitization and inhibition of ionizing radiation-induced NF-kappaB activation in HeLa cells occur via a mechanism involving p38 MAP kinase. *Cancer Res* 2001; 61: 7689-96.
- [88] Dent P, Yacoub A, Fisher PB, Hagan MP, Grant S. MAPK pathways in radiation responses. *Oncogene* 2003; 22: 5885-96.
- [89] Wang T, Hu YC, Dong S, *et al.* Co-activation of ERK, NF-kappaB, and GADD45beta in response to ionizing radiation. *J Biol Chem* 2005; 280: 12593-601.
- [90] Rabbani ZN, Mi J, Zhang Y, *et al.* Hypoxia inducible factor 1alpha signaling in fractionated radiation-induced lung injury: role of oxidative stress and tissue hypoxia. *Radiat Res* 2010; 173: 165-74.
- [91] Wu CT, Chen WC, Lin PY, Liao SK, Chen MF. Androgen deprivation modulates the inflammatory response induced by irradiation. *BMC Cancer* 2009; 9: 92.
- [92] Johnston CJ, Williams JP, Okunieff P, Finkelstein JN. Radiation-induced pulmonary fibrosis: examination of chemokine and chemokine receptor families. *Radiat Res* 2002; 157: 256-65.
- [93] Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002; 2: 933-44.
- [94] Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; 383: 787-93.
- [95] Han G, Zhang H, Xie CH, Zhou YF. Th2-like immune response in radiation-induced lung fibrosis. *Oncol Rep* 2011; 26: 383-8.
- [96] Brush J, Lipnick SL, Phillips T, Sitko J, McDonald JT, McBride WH. Molecular mechanisms of late normal tissue injury. *Semin Radiat Oncol* 2007; 17: 121-30.
- [97] Tak JK, Park JW. The use of ebselen for radioprotection in cultured cells and mice. *Free Radic Biol Med* 2009; 46: 1177-85.
- [98] Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr Med Chem* 2009; 16: 130-43.
- [99] Terasaki Y, Ohsawa I, Terasaki M, *et al.* Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. *Am J Physiol Lung Cell Mol Physiol* 2011; 301: L415-26.
- [100] Fleckenstein K, Gauter-Fleckenstein B, Jackson IL, Rabbani Z, Anscher M, Vujaskovic Z. Using biological markers to predict risk of radiation injury. *Semin Radiat Oncol* 2007; 17: 89-98.
- [101] Robbins ME, Zhao W. Chronic oxidative stress and radiation-induced late normal tissue injury: a review. *Int J Radiat Biol* 2004; 80: 251-9.
- [102] Madamanchi NR, Hakim ZS, Runge MS. Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. *J Thromb Haemost* 2005; 3: 254-67.

- [103] Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003; 112: 1776-84.
- [104] Balli D, Ustiyani V, Zhang Y, *et al.* Foxm1 transcription factor is required for lung fibrosis and epithelial-to-mesenchymal transition. *EMBO J* 2013; 32: 231-44.
- [105] Roberts AB, Tian F, Byfield SD, *et al.* Smad3 is key to TGF-beta-mediated epithelial-to-mesenchymal transition, fibrosis, tumor suppression and metastasis. *Cytokine Growth Factor Rev* 2006; 17: 19-27.
- [106] Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; 425: 577-84.
- [107] Flanders KC, Sullivan CD, Fujii M, *et al.* Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol* 2002; 160: 1057-68.
- [108] Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. *EMBO J* 1998; 17: 3091-100.
- [109] Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev* 2005; 19: 2783-810.
- [110] Masszi A, Di Ciano C, Sirokmany G, *et al.* Central role for Rho in TGF-beta1-induced alpha-smooth muscle actin expression during epithelial-mesenchymal transition. *Am J Physiol Renal Physiol* 2003; 284: F911-24.
- [111] Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL. Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. *Science* 2005; 307: 1603-9.
- [112] Hurd TW, Gao L, Roh MH, Macara IG, Margolis B. Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. *Nat Cell Biol* 2003; 5: 137-42.
- [113] Perez-Moreno M, Jamora C, Fuchs E. Sticky business: orchestrating cellular signals at adherens junctions. *Cell* 2003; 112: 535-48.
- [114] Monceau V, Pasinetti N, Schupp C, Pouzoulet F, Opolon P, Vozenin MC. Modulation of the Rho/ROCK pathway in heart and lung after thorax irradiation reveals targets to improve normal tissue toxicity. *Curr Drug Targets* 2010; 11: 1395-404.
- [115] Haase MG, Klawitter A, Geyer P, *et al.* Sustained elevation of NF-kappaB DNA binding activity in radiation-induced lung damage in rats. *Int J Radiat Biol* 2003; 79: 863-77.
- [116] Anscher MS, Thrasher B, Zgonjanin L, *et al.* Small molecular inhibitor of transforming growth factor-beta protects against development of radiation-induced lung injury. *Int J Radiat Oncol Biol Phys* 2008; 71: 829-37.
- [117] Flechsig P, Dadrich M, Bickelhaupt S, *et al.* LY2109761 attenuates radiation-induced pulmonary murine fibrosis via reversal of TGF-beta and BMP-associated proinflammatory and proangiogenic signals. *Clin Cancer Res* 2012; 18: 3616-27.
- [118] Hakenjos L, Bamberg M, Rodemann HP. TGF-beta1-mediated alterations of rat lung fibroblast differentiation resulting in the radiation-induced fibrotic phenotype. *Int J Radiat Biol* 2000; 76: 503-9.
- [119] Li Y, Song LW, Peng RY, *et al.* [Effects of SB203580 and WP631 on Smad signal transduction pathway in lung fibroblasts after irradiation]. *Ai Zheng* 2008; 27: 698-702.
- [120] Brickey WJ, Neuringer IP, Walton W, *et al.* MyD88 provides a protective role in long-term radiation-induced lung injury. *Int J Radiat Biol* 2012; 88: 335-47.
- [121] Wang LH, Liu JS, Ning WB, *et al.* Fluorofenidone attenuates diabetic nephropathy and kidney fibrosis in db/db mice. *Pharmacology* 2011; 88: 88-99.
- [122] Chen LX, Yang K, Sun M, *et al.* Fluorofenidone inhibits transforming growth factor-beta1-induced cardiac myofibroblast differentiation. *Pharmazie* 2012; 67: 452-6.
- [123] Pan J, Su Y, Hou X, *et al.* Protective effect of recombinant protein SOD-TAT on radiation-induced lung injury in mice. *Life Sci* 2012; 91: 89-93.
- [124] Koukourakis MI, Panteliadou M, Abatzoglou IM, Sismanidou K, Sivridis E, Giatromanolaki A. Postmastectomy hypofractionated and accelerated radiation therapy with (and without) subcutaneous amifostine cytoprotection. *Int J Radiat Oncol Biol Phys* 2013; 85: e7-13.
- [125] Przybyszewska M, Miloszevska J, Rzonca S, *et al.* Soluble TNF-alpha receptor I encoded on plasmid vector and its application in experimental gene therapy of radiation-induced lung fibrosis. *Arch Immunol Ther Exp (Warsz)* 2011; 59: 315-26.
- [126] Oikonomou N, Harokopos V, Zalevsky J, *et al.* Soluble TNF mediates the transition from pulmonary inflammation to fibrosis. *PLoS One* 2006; 1: e108.
- [127] Wolthuis EK, Vlaar AP, Choi G, *et al.* Recombinant human soluble tumor necrosis factor-alpha receptor fusion protein partly attenuates ventilator-induced lung injury. *Shock* 2009; 31: 262-6.
- [128] Sandalon Z, Bruckheimer EM, Lustig KH, Rogers LC, Peluso RW, Burstein H. Secretion of a TNFR: Fc fusion protein following pulmonary administration of pseudotyped adeno-associated virus vectors. *J Virol* 2004; 78: 12355-65.
- [129] Walter N, Collard HR, King TE, Jr. Current perspectives on the treatment of idiopathic pulmonary fibrosis. *Proc Am Thorac Soc* 2006; 3: 330-8.
- [130] Sekine I, Sumi M, Ito Y, *et al.* Retrospective analysis of steroid therapy for radiation-induced lung injury in lung cancer patients. *Radiother Oncol* 2006; 80: 93-7.
- [131] Fujino N, Kubo H, Suzuki T, *et al.* Administration of a specific inhibitor of neutrophil elastase attenuates pulmonary fibrosis after acute lung injury in mice. *Exp Lung Res* 2012; 38: 28-36.
- [132] Kong FM, Ao X, Wang L, Lawrence TS. The use of blood biomarkers to predict radiation lung toxicity: a potential strategy to individualize thoracic radiation therapy. *Cancer Control* 2008; 15: 140-50.