Advances in Molecular Mechanisms and Treatment of Radiation-Induced Pulmonary Fibrosis

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Abstract

Radiation-induced pulmonary fibrosis (RIPF) is a common complication in patients with lung cancer and breast cancer after receiving thoracic radiotherapy. The average incidence of RIPF is 16%-28% after radiotherapy. RIPF includes a heterogeneous group of lung disorders characterized by progressive and irreversible destruction of lung architecture and disruption of gas exchange. The clinical signs of RIPF include increasing dyspnea, deteriorating lung function, and accumulation of interstitial fluid, eventually leading to respiratory failure. No medical therapy for RIPF has been approved for routine clinical use despite the apparent need for an effective treatment. Numerous signaling pathways are involved in the initiation and progression of RIPF. Also, various approaches for RIPF treatments have focused on several aspects of the current understanding of the molecular pathology of RIPF. This review used the mechanistic categories of associated cell signaling pathways, epithelial cell dysfunction and senescence, abnormal lung remodeling, and aberrant innate and adaptive immunity to review the published literature on RIPF to date and then to identify potential areas for the effective treatment of RIPF.

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Introduction

Lung cancer is the leading cause of cancer death all over the world. Its overall 5-year survival rate is only approximately 15%. Although the best outcomes are achieved with surgery, more than 64.3%±4.7% of patients with non–small cell lung cancer require radiation therapy at least once because of medical comorbidities or extensiveness of the disease [1]. Irrespective of the extensive use of stereotactic radiotherapy, which limits the exposure of normal lung tissue to irradiation, as many as 35% of patients with lung cancer and breast cancer receiving thoracic radiotherapy develop radiation pneumonitis and are at strong risk of developing radiation-induced pulmonary fibrosis (RIPF) months and years after initial radiotherapy [2–4]. According to old radiation therapy oncology group data, the average incidence of RIPF was 28% after two-dimensional radiotherapy. Cella et al. reported an overall RIPF incidence of 16% (8% symptomatic and 8% asymptomatic) in 115 patients with Hodgkin's lymphoma who received three-dimensional radiotherapy [5]. RIPF includes a heterogeneous group of lung disorders characterized by progressive and irreversible destruction of lung architecture and disruption of gas exchange [6]. The clinical signs of RIPF include increasing dyspnea, deteriorating lung function, and accumulation of interstitial fluid, eventually leading to respiratory failure. While steroids and other forms of anti-inflammatory therapy have been established to control acute pulmonary inflammation, no medical therapy for RIPF has been approved for routine clinical use despite the apparent need for an effective treatment [7].

Prevailing hypotheses suggest that RIPF is an epithelial-fibroblastic disorder. Ionizing radiation injures pulmonary epithelial and endothelial cells and causes the release of proinflammatory cytokines that recruit macrophages and lymphocytes to the sites of injury [6].

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Activated myofibroblasts play a central role during the pathogenesis of pulmonary fibrosis by synthesizing and depositing extracellular matrix (ECM) proteins. Myofibroblasts are likely derived from various cells, including 1) resident stromal fibroblasts, 2) bone marrow–derived "fibrocytes," and 3) alveolar type II epithelial cells, a subset of which undergoes epithelial-to-mesenchymal transition (EMT) [8]. During EMT, epithelial cells lose apical-basal polarity, basement membrane attachment, and cell-cell contact. They gain mesenchymal characteristics associated with increased migratory behavior, cytoskeletal rearrangements, and their migration into the lung interstitium where they produce excess ECM. During the normal healing process, alveolar-capillary permeability is restored and inflammation is resolved. Radiation to the thorax causes pulmonary fibrosis as lung injury, inflammation, and remodeling persist [9]. Numerous signaling pathways are involved in the initiation and progression of RIPF. Also, various approaches for RIPF treatments have focused on several aspects of the current understanding of the molecular pathology of RIPF.

This study used the mechanistic categories of associated cell signaling pathway, epithelial cell dysfunction and senescence, abnormal lung remodeling, and aberrant innate and adaptive immunity to review the published literature in RIPF to date and then to identify potential areas for the effective treatment of RIPF.

Associated Cell Signaling Pathway

Latency-associated peptide (LAP) interacts with proteins of the latent transforming growth factor-β (TGF-β) binding protein family, which anchor latent TGF-β to the extracellular matrix [10]. LAP and TGF-β are secreted as a complex in which TGF-β is latent. The release of TGF-β from LAP is a highly regulated step in TGF-β signaling [11]. TGF-β is a multifunctional regulator of cell growth, EMT, and differentiation in response to injuries [12–14]. The expression of TGF-β induced by radiation has been reported as part of RIPF [15–17]. Once the ligand/receptor complex of TGF-β is formed, intracellular effector molecules are phosphorylated by the receptor to induce numerous intracellular pathways [18]. Hypoxia-inducible factor -1α (HIF-1α) can affect the irradiation-induced endothelial-to-mesenchymal transition (EndMT) via the TGFβ-R1/Smad3 signaling pathway [19]. HIF-1α siRNA inhibits radiation-induced EndMT accompanied by a decrease in TGFβ-R1/Smad3 signaling [20–22]. Serine palmitoyltransferase (SPT) catalyzes the first step in the chain of reactions leading to the formation of complex sphingolipids, determines the rate of de novo sphingolipid biosynthesis [23], and provides increased sphingoid base supply affecting dihydrosphingosine-dihydroceramide levels. Targeting SPT decreases sphingosine-kinase-1 (SphK1) activity in the lung, reduces the levels of sphingosine-1-phosphate (S1P) and dihydro-sphingosine-1-phosphate (DHS1P) in the lung and circulation, and delays the onset of RIPF [24].

The platelet-derived growth factor (PDGF)-PDGFR system is involved in idiopathic pulmonary fibrosis (IPF) and asbestos, bleomycin, and RIPF [25], besides fibrosis in other organs such as the kidneys, liver, skin, and heart [26,27]. During fibrosis, the PDGF-PDGFR system may be a promising target for treating fibrotic disease [28]. The role of TGF-β and PDGF signaling in the development of pulmonary fibrosis has been well established [29,30]. Also, the upregulation of components of the TGF-β and PDGF signaling cascades was reported in lung tissues after thoracic RT [15,31]. The development of radiation-induced fibrosis was attenuated by blocking either TGF-β or PDGF effects in animal models [31,32].

Cannabinoid receptor-1 (CB1) is known to be expressed in the lung tissue, bronchial epithelial cells, and alveolar type II cells. Activation of CB1 may exert proinflammatory or pro-oxidant effects, further leading to RIPF [33,34].

Ecto-5'-nucleotidase (CD73) acts in concert with ectonucleoside triphosphate diphosphohydrolase 1 (CD39) to generate adenosine from extracellularly released ATP/ADP. Extracellular adenosine acts through four different G protein–coupled adenosine receptors that are widely expressed and have various biological functions aimed at maintaining or restoring tissue homeostasis. Both CD73 and adenosine are critical in balancing tissue inflammation and repair processes in pulmonary fibrosis; regulating leukocyte extravasation and function; and modulating epithelial cell behavior, vascular function, and cell death [35].

Epithelial Cell Dysfunction and Senescence

Airway epithelial cell II (AEC II) can function as the alveolar stem cell, proliferating in response to injury and differentiating into both AEC I and AEC II [36]. AEC II depletion is hypothesized to be a major contributor to ineffective alveolar repair [37,38], leading to epithelial stress and fibroproliferation [39]. Extensive AEC II loss stimulates macrophage influx and proinflammatory cytokine elaboration, resulting in fibrosis [38,40]. The severity of AEC II depletion correlates with the degree of senescence [41]. Senescent cells secrete proinflammatory cytokines, such as interleukin 6, TGF-β, and interleukin 1-α, which are implicated in RIPF [42,43]. A time- and dose-dependent increase of AEC II senescence and pneumocyte depletion after exposure to fibrosis-inducing doses of irradiation was reported [41]. AEC II senescence is involved in RIPF and may serve as a novel target for intervention (Figure 2).

Superoxide dismutase (SOD) belongs to a family of metalloprotein enzymes important in protection from oxidative damage. Several SOD gene therapy studies in animals have suggested the protective effect of SOD against radiation toxicity in the lung [44]. Extracellular SOD (EC-SOD) is the predominant antioxidant enzyme; in the lung, it is primarily located to type II pneumocytes and macrophages. The overexpression of EC-SOD in transgenic mice confers protection against RIPF, with a corresponding decrease in oxidative stress [45].

Abnormal Lung Remodeling

The numbers of cells undergoing EMT during pulmonary fibrosis were estimated to be between 5% and 20% depending on the mouse model or human samples [46–48]. EMT is controlled by a network of signaling and transcriptional events mediated in part by TGF-β signaling. Snail and Twist family members of transcription factors are also important regulators of EMT, repressing E-cadherin and activating the mesenchymal transcriptomes [49]. Snail1 can induce EMT and the expression of EMT-associated genes, and may act as a switch to promote EMT program in epithelial cells [50]. EMT is also controlled by the Wnt/β-catenin pathway. Further, aberrant activation of β-catenin has been demonstrated in idiopathic pulmonary fibrosis [50–53]. Regulatory T cells (Tregs) contribute to RIPF though promotion of EMT and β-catenin accumulation in the alveolar epithelium. They also accelerate EMT partly through β-catenin [54].

Myofibroblasts are likely derived from bone marrow (BM)–derived fibroblast progenitor cells that are crucial in the fibrotic process [55] (Figure 2). The BM-derived fibrocytes express a chemokine receptor CXCR4. Activation of the CXCR4 by its ligand stromal cell–derived factor 1 (SDF-1/CXCL12) may be important in the development of

RIPF. Therefore, the CXCR4/CXCL12 axis is critical in recruiting BM-derived precursors that differentiate into the fibroblasts that cause RIPF.

Matrix metalloproteinases (MMPs) constitute a family of extracellular zinc- and calcium-dependent proteases that degrade ECM and other extracellular proteins, the extensive remodeling of which can result in fibrogenesis [56] (Figure 2). MMP13 is a highly specific interstitial collagenase in rodents capable of degrading insolubale fibrillar collagens [56]. MMP13 is also a key activator of a whole cascade of proinflammatory reactions in the lungs. The presence of MMP13 may cause an increase of ECM in mouse lungs [57].

Intercellular adhesion molecule 1 (ICAM-1) and E-selection are two cell adhesion proteins located on endothelial cells of the microvasculature. Leukocytes subsequently adhere to the endothelial cells via these adhesion molecules. ICAM-1$^{-/-}$ mice showed no inflammatory cell infiltration of their lungs after thoracic irradiation [58]. Also, irradiated ICAM-1$^{-/-}$ mice had lower levels of hydroxyproline in their lungs and improved pulmonary compliance than did irradiated ICAM$^{+/+}$ mice. The improvement in pulmonary compliance accounted for the attenuation of RIPF.

Matricellular protein connective tissue growth factor (CTGF) not only is an essential mediator for the fibrotic activity of TGF-β but also can act independently of TGF-β. It can modulate the formation of myofibroblasts by regulating the transdifferentiation of fibroblasts or epithelial cells or by enabling edema leading to the deposition of provisional matrix on which the epithelial cells undergo EMT. CTGF stimulates myofibroblasts to express chemokines and cytokines that recruit leukocytes and regulate their activity and to deposit and remodel ECM, leading to changes in organ structure and function [59].

Aberrant Innate and Adaptive Immunity

Thorax irradiation triggered the recruitment of various immune cells into the lung. Preclinical and clinical studies have shown that activated T lymphocytes constitute a significant part of immune cells infiltrating the lung tissue on thorax irradiation; they are also an important cellular component involved in the control of RIPF. Naive CD4+ T-helper (Th0) cells can be differentiated into at least two functional subsets during the immune response: Th1 cells (type 1), which secrete Th1 cytokines such as INF-γ, TNF-β, IL-2, and IL-12, and Th2 cells (type 2), which secrete Th2 cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13 [60]. The differentiation of Th0 cells into Th1 or Th2 cells is regulated by the following transcription factors: T-box expressed in T cells (T-bet) and GATA-binding protein-3 (GATA-3). The expression of Th2-related cytokines increased and that of Th1-related cytokines decreased after radiation exposure. Then, alveolar macrophage accumulation increased in the irradiated tissue, and TGF-β was expressed at a higher level. In TGF-β-Smad–dependent pathways, the expression of TGF-β1, TβR, and phosphor-Smad 2/3 was upregulated and the expression of Smad 7 was downregulated, leading to the development of RIPF [61]. Han et al. reported that Th2-like immune response was involved in RIPF, and GATA-3 had an important role in promoting RIPF [62]. Irradiation can induce lung fibrosis in tumor-bearing animals more easily than in healthy mice. This may be because tumors deliver type 2 cytokines or other profibrogenic signals into normal lung tissue via the circulation, eventually leading to RIPF [63]. The signaling of Toll-like receptors (TLRs) can influence innate and adaptive immune responses [64,65]. The activation of TLR-9 in part regulates the type 1/type 2 immune environment in irradiated lung tissue by enhancing type 1 immunity

and alleviates RIPF [66]. The activation of TLR-5 has also been reported to be quite effective in protecting cells, mice, and nonhuman primates against radiation-induced lethality [67,68].

The bronchoalveolar lavage of patients after radiotherapy contained increased numbers of mast cells and neutrophils [69]. Animal models of RIPF also showed an increase in the levels of tissue mast cells and lavage neutrophils [70]. Inflammatory monocytes, which are derived from the bone marrow and express CCR2, were shown to migrate into the lung after radiation exposure. CCR2$^+$ infiltrating monocyte-derived macrophages played a critical role in the development of RIPF [71].

Potential Areas for Effective Treatment of RIPF

Integrin vβ6 is a major TGF-β activator in the lung, which interacts with the amino acid sequence arginine-glycine-aspartic acid (RGD) located near the C terminus of LAP, a peptide derived from the N-terminal region of the TGF-β gene product that is part of the TGF-β-LAP complex [72] (Figure 1). The vβ6-mediated TGF-β activation was a major contributor to fibrosis [73]. Also, low-level doses of vβ6 inhibition prevented fibrosis without altering bronchoalveolar lavage (BAL) cell counts or the levels of selected inflammatory mediators. Direct inhibition of vβ6 and prevention of its upregulation are potentially useful strategies to treat or prevent RIPF. LY2109761 is a novel small-molecule TGF-β receptor I serine/threonine kinase inhibitor (Figure 1). It not only attenuated profibrotic signaling directly but also balanced the complex TGF-β/bone morphogenetic proteins (TGF-β/BMP) signaling [32]. Moreover, it reduced the radiation-induced expression of downstream signaling genes of TGF-β, including Smads 6, 7, and 9 and MDM2. Galunisertib is a highly selective inhibitor of TGFβR1 that has been demonstrated to completely inhibit the phosphorylation and activation of several downstream targets of the receptor [74] (Figure 1). 2-Methoxyestradiol (2-ME), a metabolite of 17-beta-estradiol, has been suggested to effectively inhibit the action of HIF-1α (Figure 1). 2-ME inhibited the radiation-induced increase in the expression of HIF-1α *in vivo*, leading to a decrease in EndMT and concomitant deposition of vascular collagen during the development of RIPF. 2-ME also reduced EMT and the fibrotic phase [19]. Hence, 2-ME may be used to prevent RIPF. Myriocin is a specific inhibitor of SPT. It can block the *de novo* biosynthesis of sphingolipids. Targeting SPT with myriocin can counteract TGF-β–induced signaling, defer radiation-induced SphK1 activation and RIPF progression, and serve as a novel therapeutic target for managing RIPF. Syndecan-2 attenuated pulmonary fibrosis in mice exposed to radiation and inhibited TGF-β1–induced fibroblast-myofibroblast differentiation, migration, and proliferation by downregulating P13K/Akt/ROCK signaling and blocking the binding of serum response factor to α-SMA promoter via CD148. It can be used as an antifibrotic therapy in RIPF [75]. PDGF receptor tyrosine kinase inhibitors (Imatinib/Gleevec, SU9518, and SU11657) markedly attenuated the development of fibroblast foci, the hallmark of pulmonary fibrosis, and the subsequent remodeling of the lung architecture (Figure 1). It is unlikely that single pathway inhibition can completely prevent lung fibrosis in humans, considering the intricate genetic networking [76,77]. However, the PDGF receptor tyrosine kinase inhibitors are undergoing clinical trials and may have appropriate potency, selectivity, and safety profiles for treating RIPF. SU9518 is a highly selective inhibitor of PDGF receptors α and β. It has been shown to block PDGFR kinase activity and PDGFR-induced cellular
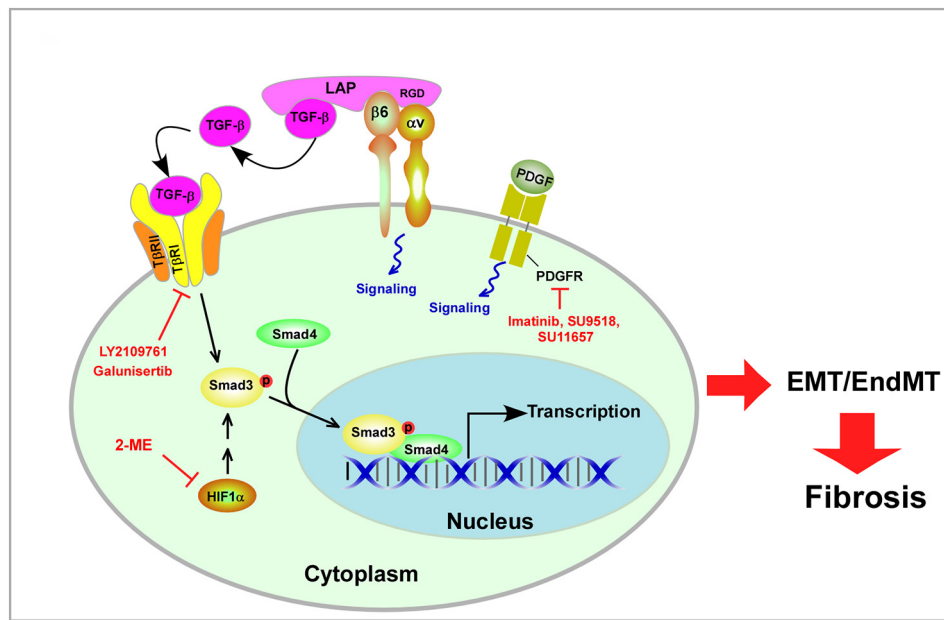
Figure 1. Associated cell signaling pathway and related potential therapeutic areas. Integrin avβ6 interacts with the amino acid sequence RGD, which is located near the C-terminus of LAP. TGF-β can be activated after release from LAP. The inhibition of avβ6-mediated TGF-β activation prevents RIPF. Both LY2109761, a small molecule TGF-β receptor 1 serine/threonine kinase inhibitor, and galunisertib, a highly selective inhibitor of TGFβR1, attenuate RIPF by inhibiting TGF-β–associated downstream targets. Further, 2-methoxyestradiol (2-ME) effectively inhibits the action of HIF-1α, reducing EndMT, EMT, and concomitant deposition of vascular collagen and eventually attenuating the development of RIPF. PDGF receptor tyrosine kinase inhibitors (imatinib, SU9518, and SU11657) markedly attenuated the development of fibroblast foci and subsequent remodeling of lung architecture.

proliferation [78,79]. SU14816 has activity against c-Kit and PDGF receptors α and β (Figure 1). Animal studies demonstrated that the dual-pathway blockade showed additional benefits regarding the development of pulmonary fibrosis and mouse survival on using Galunisertib, SU9518, and SU14816 after thoracic irradiation compared with the inhibition of each pathway alone [80]. AM6545, a pharmacological inhibition of CB1, not only significantly attenuated RIPF but also prolonged the survival of animals. AM6545 can selectively affect CB1-mediated signaling in peripheral organs without affecting neurotransmission in the central nervous system because of its lower brain/plasma ratio. Pegylated adenosine deaminase (PEG-ADA) can be used to decrease tissue adenosine levels. PEG-ADA and targeting CD73 with monoclonal antibody TY/23 significantly reduced RIPF. Modulating adenosine may be effective in limiting RIPF [35]. More recently, molecular advances on radiation-induced fibrogenesis showed an essential role in epigenetic regulation. The bromodomain and extra-terminal inhibitor JQ1 attenuated radiological and histological presentations of RIPF [81].

NADPH oxidase (NOX) is a critical mediator of radiation-induced AEC II senescence and pulmonary fibrosis [41]. Diphenyleneiodonium (DPI), an inhibitor of NOX, was sufficient to prevent AEC II senescence and markedly reduced RIPF (Figure 2). The mTOR signaling pathway is known to play an essential role in aging and senescence. The signaling of mTORC 1 is rapamycin sensitive and thought to mediate the effects of the pathways on aging and senescence through p70S6 kinase (p70S6K) or S6 kinase 1 (S6K1) [82,83]. Treatment with rapamycin resulted in inhibition of radiation-induced signaling downstream of mTOR; reduced the expression of profibrotic, proinflammatory, and senescence-associated cytokines in irradiated lungs; exhibited a marked reduction in

macrophage accumulation, collagen content, and AEC II senescence; reduced RIPF; and significantly prolonged survival after lethal thoracic irradiation in C57BL/6NCr mice [84] (Figure 2). SOD protected normal lung tissue from radiation by decreasing oxidative stress, thereby attenuating RIPF with the same dose of radiation therapy. Amifostine, a synthetic sulfhydryl compound, plummeted the accumulation of reactive oxygen species (ROS) and macrophages and decreased the expression of TGF-β1 in irradiated lung tissues of rats. It is the only drug approved by the US Food and Drug Administration as a radioprotector [85]. Cytochrome P450 2E1 (CYP2E1) regulated the levels of endoplasmic reticulum stress and ROS in alveolar epithelial type II (AE2) cells and lung fibroblasts. Inhibition of CYP2E1 significantly attenuated EMT transition, apoptosis of AE2 cells, and myofibroblast formation [86].

Forkhead box M1 (FOXM1) is a member of the Forkhead family of transcription factors that share homology in the Winged Helix/ Forkhead box DNA-binding domain. Conditional deletion of FOXM1 from alveolar type II cells and siRNA-mediated depletion of FOXM1 from cultured A459 cells can decrease the expression of Snail1 mRNA and protein and then attenuate RIPF by repressing EMT (Figure 2). As a subset of CD4$^+$ T lymphocytes, Tregs express the surface marker CD25 and the transcription factor Foxhead box protein 3 (Foxp3). The anti-CD25–directed antibody is always used specifically to deplete Tregs [87]. The monoclonal anti-CD25 antibody treatment was found to partially attenuate RIPF probably through inhibiting the involvement of Tregs in immune response [54]. MSX-122 is a novel small molecule and partial CXCR4 antagonist. It was discovered as one of the most potent CXCR4 inhibitors with reasonable bioavailability and has been considered as a clinical drug candidate. It displays high-affinity binding to CXCR4
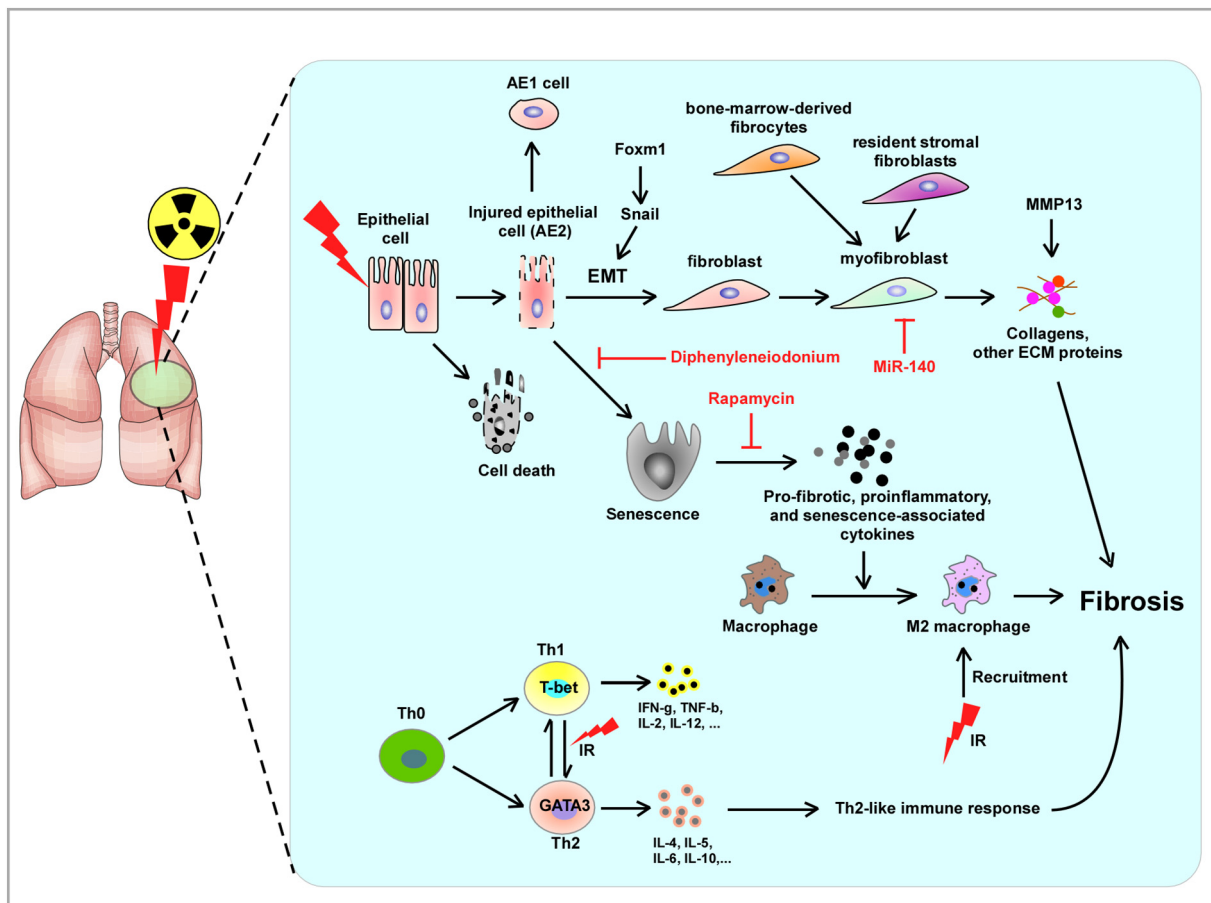
Figure 2. Epithelial cell injury, abnormal lung remodeling, and related potential therapeutic areas. Diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase (NOX), prevents AECII senescence and markedly reduces RIPF. Rapamycin inhibits radiation-induced signaling downstream of mTOR; reduces expression of profibrotic, proinflammatory, and senescence-associated cytokines in irradiated lungs; and prevents RIPF. Forkhead box M1 (Foxm1) can decrease the expression of Snail1 mRNA and protein and attenuate RIPF by repressing EMT. MiR-140 inhibits myofibroblast differentiation and inflammation and prevents RIPF. MMP13 is relevant with ECM deposition and remodeling of lung architecture. MMP13 knockouts dramatically reduce lung density and shrinkage of lung volume and attenuate RIPF. GATA-3 develops type 2 phenotype and produces type 2 cytokines while inhibiting Th1 cells. Inhibition of GATA-3 attenuates RIPF.

and inhibition of receptor function in the sub-nM range without metal-chelating capability [88]. MSX-122 can alleviate RIPF in a mouse model [89]. The possible use of MSX-122 for blocking RIPF among patients undergoing thoracic irradiation warrants further investigations. MMP13 knockouts dramatically reduced lung density and shrinkage of the lung volume, directly reducing lung fibrosis. MMP13 is more relevant in the later fibrogenesis phase with ECM deposition, remodeling of the architecture, and lung fibrosis than in the acute pneumonitis phase [57]. MMP13 can be a potential drug target to attenuate RIPF. Mice with a homozygous null mutation in the ICAM-1 gene had less pulmonary fibrosis and reduced thickening of the alveolar septum. Blocking ICAM-1 function or expression should be explored for its effects on the prevention of RIPF. FG-3019, a human monoclonal antibody to CTGF, reprogrammed fibrogenesis via normalization of the radiation-induced expression of genes involved in inflammation associated with M2 macrophage influx, EMT, myofibroblast activation, remodeling, and ECM deposition. FG-3019 reduced fibrotic disease activity by normalizing radiation-induced SMA and the expression of osteopontin protein and mRNA. Transient administration of FG-3019 to irradiated mice prevented and reversed lung remodeling, preserved lung function,

and provided a survival benefit [59]. The loss of miR-140 is a marker of fibrotic lung tissue *in vivo*. MiR-140 knockout primary lung fibroblasts have a higher percentage of myofibroblasts, and miR-140 deficiency promotes the accumulation of M2 macrophages in irradiated lung tissues. MiR-140 serves a key protective molecule against RIPF through inhibiting myofibroblast differentiation and inflammation [90].

CpG-oligodexynucleotide (ODN), a nonmethylated, short, single-stranded synthetic DNA molecule, is a TLR ligand [91]. CpG-ODN treatment following irradiation significantly increased the expression of type 1 inflammatory cytokines (IL-12 and INF-γ) and decreased type 2 inflammatory cytokines (IL-13 and IL-5) and TGF-β. It also attenuated RIPF by reversing the type 2 immunosuppressive microenvironment in fibrotic renal tissue [92]. Meanwhile, CpG-ODN reduced the injury caused by ROS in mice after irradiation, thereby alleviating RIPF [93].

TLR activation requires a host of intracellular adaptor proteins proximal to TLR, with the most prominent being myeloid differentiation primary response factor 88 (MyD 88) [94]. MyD 88 contains two prominent domains, the Toll-like receptor/interleukin 1 receptor domain and a death domain [95]. MyD 88 is a key control element for innate

immune signaling in response to pathogenic stimuli and mechanical and environmental stresses [96–99]. MyD 88 supported survival from radiation-induced injury through regulating inflammatory factors that aided in recovery from irradiation [100]. The absence of MyD 88 resulted in unresolved pulmonary infiltrates, enhanced collagen deposition, and elevated levels of Th2 cytokines in long-term survivors of irradiation.

Natural resources may be a better choice for attenuating RIPF due to their multitargeted activity. Podophyllotoxin possesses the properties of cell-cycle arrest in the G2/M phase, regulation of DNA repair pathway, and cellular proliferation in both *in vitro* and *in vivo* model systems. Rutin demonstrates strong proton-donating and free radical–stabilizing properties. A formulation (G-003M) prepared by combining two molecules podophyllotoxin and rutin was reported to modulate immunity and inhibit RIPF by attenuating oxidative/ nitrosative stress and downregulating the expression of inflammatory/ fibrogenic cytokines [101].

Conclusions

No treatment method for RIPF has been approved for routine clinical use to date despite an urgent need to improve the treatment of RIPF. Many signal pathways and potential targets are involved in RIPF. They apparently attenuate fibrosis levels in RIPF models. Based on the findings, it would be plausible to presume that RIPF will be cured in the future.

However, parallel to the drug development, it would be also important to identify predictive biomarker that helps in selecting susceptible patients for administering a lower radiation dose and avoiding the occurrence of RIPF. Efforts should be made in the future to gain more knowledge on novel RIPF inhibitors so as to improve the treatment of patients with RIPF.

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Acknowledgements

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