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The Cellular and Molecular Mechanism of Radiation-Induced Lung Injury

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The lung is one of several moderately radiosensitive organs. Radiation-induced lung injury (RILI), including acute radiation pneumonitis and chronic radiation-induced pulmonary fibrosis, occurs most often in radiotherapy of lung cancer, esophageal cancer, and other thoracic cancers. Clinical symptoms of RILI include dry cough, shortness of breath, chest pain, fever, and even severe respiratory failure and death. The occurrence of RILI is a complex process that includes a variety of cellular and molecular interactions which ultimately leads to large fibroblast accumulation, proliferation, and differentiation, resulting in excessive extracellular matrix deposits, causing pulmonary fibrosis. The progress that has been made in recent years in the understanding of cellular and molecular mechanisms of RILI is summarized in this review.

MeSH Keywords: Abnormalities, Radiation-Induced • Fibroblasts • Macrophage Activation • Transforming Growth Factor beta1

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Background

There are a series of cellular and molecular changes that occur when the lung tissue suffers ionizing radiation that does not cause immediate clinical symptoms. Pulmonary irradiation can produce a lot of reactive oxygen species and reactive nitrogen which causes oxidative damage of DNA, lipid, and protein. The resulting injury or apoptosis of alveolar epithelial cells and vascular endothelial cells then induce a series of inflammatory reactions and chemotaxis of monocytes, lymphocytes, and granulocytes, which gather at the site of tissue injury. The result is secretion of large amounts of inflammatory cytokines, chemokines, and growth factors, such as TGFβ, IFN-γ, ET-1, IL4, IL-13, which aggregate more inflammatory cells. When this damage becomes chronic, it ultimately leads to pulmonary fibrosis. In summary, a variety of cells and molecules are involved in the complex process of radiation-induced lung injury (RILI) (Figure 1).

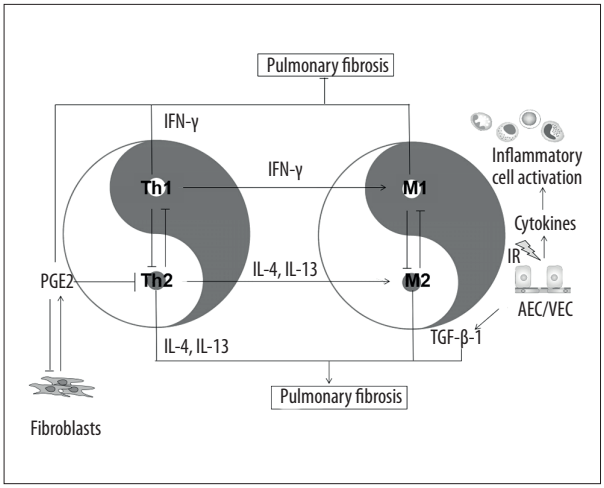

Figure 1. When lung tissue suffers ionizing radiation, alveolar epithelial cells (AEC) and vascular endothelial cells (VEC) are damaged, and then secrete large amounts of cytokines, which induce acute radiation pneumonitis. In this period, Th-1 derived IFN-γ induces activation of M1 macrophages. As a major effector cytokine of Th1 cells, IFN-γ auto-amplifies Th1 responses and cross-inhibits the differentiation and function of Th2 cells and the expression of Th2-derived cytokines IL-4 and IL-13, which induce activation of M2 macrophages that promote fibrosis through the production of TGF-β1. Meanwhile, activated fibroblasts secret great amounts of PGE2, which, in turn, triggers a negative feedback on the production of cytokines, and inhibits the transendothelial migration of T cells and transition to Th2 cells, and downregulates the functions of fibroblasts, including proliferation, collagen synthesis, and myofibroblast transformation.

Related Cells

Alveolar epithelial cells and vascular endothelial cells

Ionizing radiation-induced injuries in alveolar epithelial cells and vascular endothelial cells are important contributors to RILI and the resulting chronic progression condition. When alveolar epithelial cells and vascular endothelial cells are injured, the connections between cells is damaged, accompanied by impairment on the regulation of myofibroblast, so that excessive extracellular matrix deposits result in radiation-induced pulmonary fibrosis [1]. Once the barrier of lung tissue is damaged, a large number of blood exudate and inflammatory cells accumulate in the alveolar cavity, which then aggregates numerous fibroblasts and induces their differentiation into myofibroblasts. Activated myofibroblasts then secret angiotensin and hydrogen peroxide, which in turn induces apoptosis of alveolar epithelial cells [2]. The damage to alveolar epithelial cells and vascular endothelial cells results in secretion of a large number of pro-inflammatory and pro-fibrotic cytokines, including TGF-β1, IL-13, ET-1, PGE2 [3].

Th1 and Th2 cells

Th1/Th2 imbalance plays an important role in the development of RILI. When lung tissue is exposed to ionizing radiation, Th1 cells play a role in RILI mainly through the secretion of IFN-γ, whereas Th2 cells play a role mainly through the secretion of IL-4 and IL-13. IFN-γ increases early after irradiation, while Th2-derived IL-4 and IL-13 do not significantly increase in the early stage, but gradually increases over time and are maintained at a high level [4]. IL-4 and IL-13 can co-stimulate with TGF-β1 in collagen synthesis, playing significant roles in tissue remodeling and fibrosis [5,6], while Th1-derived IFN-γ have significant anti-fibrosis and immunomodulatory effects [7]. Excessive Th1 immune response mostly contributes to acute radiation pneumonitis, whereas excessive Th2 immune response mostly contributes to chronic radiation-induced pulmonary fibrosis.

Macrophages and fibroblasts

Interleukins, tumor necrosis factor, transforming growth factor, and platelet-derived growth factor induce activation of macrophages and fibroblasts, which are the major effector cells for synthesis of extracellular matrix [7]. Macrophages can be divided into two types, depending on the induction of cytokines from activated Th1 cells or Th2 cells. Th1-derived cytokine IFN-γ can promote the expression of nitric oxide synthase of macrophages, which is known as classic activated macrophages (M1). And Th2-derived cytokines IL-4 and IL-13 can promote the activity of arginase in macrophages, which is called bypass activated macrophages (M2) [8,9]. In addition, dendritic cells [10] and fibroblasts [11] will have a similar effect when

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subjected to Th1/Th2-derived cytokines. The synthesis of proline could be eventually promoted by the arginase pathway, and proline is the necessary substance for synthesis of collagen, which is the main component of the extracellular matrix, mainly synthesized and secreted by myofibroblasts. Fibroblasts and myofibroblasts accumulate in three ways: *in situ* proliferation, epithelial-mesenchymal transformation, and derivation from bone marrow. Accumulation of fibroblasts and myofibroblasts is induced by TGF-β1, PDGF, CXCL12, and other factors, of which the most important is TGF-β1 [12]. A variety of profibrotic cytokines (such as IL-4, IL-13, and TNF-α) and growth factors (such as PDGF and CTGF) connect directly or indirectly with TGF-β1. In the normal repair process, the myofibroblasts shrink the damaged area, and then epithelial cells and endothelial cells divide and migrate to repair the epithelium and endothelium. However, when the tissue suffers sustained injury, chronic inflammation and abnormal repair processes can lead to excessive secretion and deposition of extracellular matrix, and ultimately lead to fibrosis and structural changes.

Related Molecules

TGF-β

TGF-β is a potent pro-fibrotic growth factor [13], and its roles are mainly as follows: first it induces proliferation and differentiation of fibroblasts; second it promotes synthesis of collagen by fibroblasts and inhibits synthesis of collagenase and plasminogen activator; and third it aggregates a variety of inflammatory cells and promotes release of PDGF, TNF-α, IL-4, IL-6, IL-13, etc. There are many subtypes of TGF-β, among which TGF-β1, mainly generated by macrophages [14], is mainly associated with fibrosis. In the model of bleomycin-induced pulmonary fibrosis, almost all active TGF-β1 is generated by alveolar macrophages [15]. TGF-β1 is often combined with latency-associated peptide (LAP) in an inactive form [16]. Therefore, the release and activation of TGF-β1 is indispensable for its combination with the receptors TGFBR2 and ALK5, and for the following signal transduction process. Smad2/3, a cytoplasmic effector molecule of TGF-β1, phosphorylates and combines with Smad4, and then translocate into the nucleus to regulate transcription of target genes, including those encoding type I and type III collagen [17]. However, macrophage-derived TGF-β1 often promotes fibrosis [18], while T cell-derived TGF-β1 may inhibit fibrosis [19]. Moreover, a small amount of active TGF-β1 may have an anti-inflammatory effect, and only a relatively large amount of active TGF-β1 has a pro-fibrotic effect [20].

ET-1

Endothelin-1 (ET-1), mainly regulated by TGF-β1 in synthesis and secretion [21], is a potent endothelial-derived 21-amino-acid vasoconstrictor peptide [22]. The expression of ET-1 is induced by TGF-β1 through the ALK5/Smad3 pathway [23]. The activation of ALK5/Smad3/ET-1 pathway inhibits the migration and proliferation of endothelial cells, and increases the expression of fibrosis-associated genes, such as type I collagen and plasminogen activator inhibitor (PAI-1). It has been confirmed that ET-1 is not only a vasoconstrictor, but it is also involved in a number of other physiological processes, such as extracellular matrix deposition [24]. When ET-1 is blocked, the differentiation of fibroblasts into myofibroblasts induced by TGF-β1 will be blocked [25]. In addition, the use of ET-1 antagonist can prolong the survival time of patients with idiopathic pulmonary fibrosis [25].

IL-4

IL-4 is one of the symbolic cytokines of Th2 cells, and it can also be generated by macrophages, fibroblasts and epithelial cells. IL-4 can promote the differentiation of T cells into Th2 cells and the expression of Th2-derived cytokines, and can inhibit the activities of Th1 cells. IL-4 is closely related to radiation-induced pulmonary fibrosis. In serum of patients with idiopathic pulmonary fibrosis [26] and patients with RILI [27], IL-4 is significantly increased. *In vitro*, when fibroblasts are treated by IL-4, the expression of type I collagen, type III collagen, and fibronectin increases significantly [28–30].

IL-13

IL-13 is secreted mainly by Th2 cells as well as IL-4, and it can also be generated by mast cells, basophils, and macrophages. IL-13 has many similar functions as IL-4 because they the share α chain of IL-4 receptors, which has an effect through the activation of STAT6 [31]. IL-13 is an important pro-fibrotic cytokines and it is closely related with fibrosis of liver [32], lung [33], and skin [34]. Expression of IL-13 is regulated by endogenous transcription factor GATA-3, which can promote the expression of IL-3 through combining with a promoter sequence of IL-13 [35]. Han et al. [36] studied the role of Th2 cells in radiation-induced pulmonary fibrosis, and found that GATA-3, IL-13, and Arg-1 were significantly increased.

IFN-γ

IFN-γ, secreted mainly by Th1 cells, has significant anti-fibrosis and immunomodulatory effects [7], which are associated with inhibition of IL-4, IL-13, and TGF-β1-related pathways [37]. As a major effector cytokine of Th1 cells, IFN-γ auto-amplifies Th1 responses and cross-inhibits the differentiation and function of Th2 cells and the expression of Th2-derived cytokines. In addition, IFN-γ induces the expression of Smad7, which plays an inhibitory effect, such as blocking the activation of Smad3 [38] which can also be directly inhibited when bound with IFN-γ-activated STAT1 [39].

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PGE2

Prostaglandin E2 (PGE2), derived from arachidonic acid by the catalytic action of cyclooxygenase (COX), plays the role of pro-inflammatory mediator in a variety of diseases. However, in lung tissue, PGE2 plays a unique role in limiting the inflammatory response and the process of tissue repair [40]. PGE2 can inhibit the secretion of TGF-β, the migration of T cells and their differentiation into Th2 cells, and the differentiation of fibroblast into myofibroblast. There are a variety of cytokines produced in the early stage of pulmonary irradiation resulting in large amounts of PGE2 produced by stimulated fibroblasts. In turn, PGE2 triggers a negative feedback on the production of cytokines, and downregulates the function of fibroblasts, including proliferation, collagen synthesis, and capacity of differentiation into myofibroblast [40]. In the late stage of pulmonary irradiation, with the increased differentiation of fibroblast into myofibroblast and continued damage of epithelial cells, the generation of PGE2 decreases [41,42]; this leads to sustained activation of the immune response.

In summary, when the lung tissue suffers RILI, an abnormal repair process can ultimately lead to pulmonary fibrosis, in which a variety of pro-fibrotic cytokines, such as TGF-β, ET-1, IL-4, and IL-13, are involved. Otherwise, IFF-γ and PGE-2 T play a role in the inhibition of pulmonary fibrosis.

Conclusions

When lung tissue suffers ionizing radiation, alveolar epithelial cells and vascular endothelial cells are damaged, and inflammatory mediators are released. Blood vessel dilation and increased permeability allows for efficient accumulation of blood exudate and inflammatory cells at the site of tissue injury. In the early stage of pulmonary irradiation, Th-1 derived IFN-γ induces activation of M1 macrophages. As a major effector cytokine of Th1 cells, IFN-γ auto-amplifies Th1 responses and cross-inhibits the differentiation and function of Th2 cells and the expression of Th2-derived cytokines. If the tissue-damaging irritant persists, the exacerbated inflammatory response leads to substantial lung tissue damage, after which the Th2-derived cytokines IL-4 and IL-13 drive the conversion of the immune response into an abnormal wound healing response, which is characterized by the accumulation of M2 macrophages that promote fibrosis through the production of TGF-β1. Meanwhile, cytokines stimulate the release of great amounts of PGE2 through activated fibroblasts. PGE2, in turn, triggers a negative feedback on the production of cytokines, and inhibits the transendothelial migration of T cells and the transition to Th2 cells, and downregulates the functions of fibroblasts, including proliferation, collagen synthesis, and myofibroblast transformation.

A few achievements have been made on the cellular and molecular mechanisms of RILI, while there is remarkably little progress in the development of safe and effective therapeutic strategies. Therefore, in-depth studies on the prevention and treatment of RILI should be continued.

Competing interests

The authors have no competing interests to disclose.

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