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TOPIC HIGHLIGHT

Biological basis of radiation-induced pulmonary fibrosis

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ABSTRACT

Pulmonary fibrosis is a major radiotherapy-related toxicity, that worsens patients' quality of life, eventually leading to reduced treatment doses and subsequent decreased cancer control probability. Current therapies for radiation-induced pulmonary fibrosis are largely ineffective; thus, intense research has been done to elucidate the pathogenesis of pulmonary fibrosis and develop therapeutic strategies able to mitigate this severe side effect without interfering with anticancer treatment. The development of radiation-induced pulmonary fibrosis involves multifactorial complex processes. The severity of the response to lung irradiation is influenced by the dose of radiation, the volume of the irradiated parenchyma, the simultaneous use of chemotherapy, preexisting lung diseases, and age and genetic predispositions. Moreover, ionizing irradiation activates a cascade of genetic and nongenetic events involving several cell types, such as pneumocytes, endothelial cells, myofibroblasts and immune cells. This complex radiation-induced biological response is mediated by numerous cross-talking signaling pathways and molecules, including the TGF-β system, cytokines, the NF-kB network, and free-radicals. However, despite the recent progress made in understanding the molecular and cellular mechanisms underlying pathophysiology of lung injury after radiation exposure, much remains to be learned about how the various cells and signaling pathways interact. Thus, further studies are needed to identify reliable markers and effective targets, with the aim of developing therapeutic approaches able to prevent or treat this severe condition.

Key words: Pulmonary fibrosis; Radiotherapy; Myofibroblasts; Inflammatory mediators; Oxidative stress

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INTRODUCTION

Radiation therapy is an essential part of treatment programs for multiple thoracic malignancies, along with surgery and chemotherapy. The lungs are one of the most radiosensitive organs and the risk of severe radiation-induced side effects, such as lung fibrosis, is often dose-limiting and can significantly compromise the effectiveness of radiotherapy.

The severity of the response to lung irradiation is influenced by several factors. Radiation-induced lung injury is directly related to the total dose of radiation delivered to the lung[1], to the dose per fraction [2] and to the radiation dose rate, while inversely correlates with the number of fractions into which the dose is divided[3]. Lung injury is also proportionate to the volume of the irradiated parenchyma[4]. Usually, the damage increases as the irradiated volume increases and the lung better tolerates a high dose in a small volume than a low dose to the whole lung.

Moreover, the simultaneous use of adjuvant chemotherapy, preexisting lung disease, poor pulmonary function, age and genetic predispositions have been reported as critical factors for the development of pulmonary fibrosis[5].

Usually, the clinical and histological features of radiation-induced lung disease appear weeks after treatment and are divided into early pneumonitis and late fibrosis.

Radiation pneumonitis usually develops after about 1-3 months after radiotherapy and consists of symptomatic changes such as shortness of breath, congestion, cough and fever.

The phase of chronic inflammation and fibrosis occurs more gradually, months to years after radiotherapy[6,7]. Pulmonary fibrosis is characterized by fibroblasts and myofibroblasts accumulation, inflammatory cells infiltration, vascular damage and collagen deposition, with consequent scarring and tissue retraction[6,8] and may lead to permanent respiratory failure, with impairment of oxygen transfer[9].

Current therapies for preventing or treating radiation-induced pulmonary fibrosis are largely ineffective[5,10], thus elucidating the signaling pathways underlying pulmonary fibrosis development is urgently needed for the identification of new strategies able to treat or mitigate this devastating condition.

CELLS INVOLVED IN PULMONARY FIBROSIS DEVELOPMENT

Immediately following insult due to radiation, a rapid cascade of genetic and nongenetic events is activated[11-13].

It is now well known that fibrosis is not confined to the injured site only, but it is a systemic process involving several cell types, that are recruited to sites of injury[14].

In particular, pneumocytes (both types I and II) and endothelial cells appear to be the cells most susceptible to nongenetic damage [15]. Radiation causes apoptosis and desquamation of epithelial cells from the alveolar walls, and capillary luminal dilatation and congestion, that in turn lead to an increase in vascular permeability and production of interstitial edema, which may evolve in alveolar edema. The attempt of type II pneumocytes to re-establish a functional alveolar epithelium by proliferating and differentiating into the type I cells might even result in an abnormal epithelial repair, typical of lung fibrosis[16]. In addition, radiation-induced pulmonary damage is characterized by a large inflammatory cells infiltration and an increase in collagen deposition[17,18], ultimately leading to impairment of gas exchange and lung function[19]. The pivotal cellular mediator of pulmonary fibrosis are myofibroblasts, which are the principal producers of collagen, fibronectins and other matrix molecules after injury[20-22].

Myofibroblasts can originate from a variety of sources, including resident interstitial fibroblasts, epithelial and endothelial cells, which adopt fibroblast-like properties through processes of epithelial/endothelial to mesenchymal transition (EMT/EndMT)[14,23,24], and circulating fibrocytes, fibroblast-like cells derived from bone marrow stem cells[25,26].

The infiltrating immune cells recruited following thorax irradiation, principally the alternatively activated macrophages, but also monocytes, lymphocytes, neutrophils and basophils, have been also proposed to play a central role in the pathogenesis of pulmonary fibrosis[27,28], since they secrete cytokines and chemokines that stimulate the differentiation of fibroblasts and other cells into myofibroblasts[29].

Thus, targeting inflammatory cells has been proposed as a therapeutic strategy for radiation-induced lung injuries. For instance,

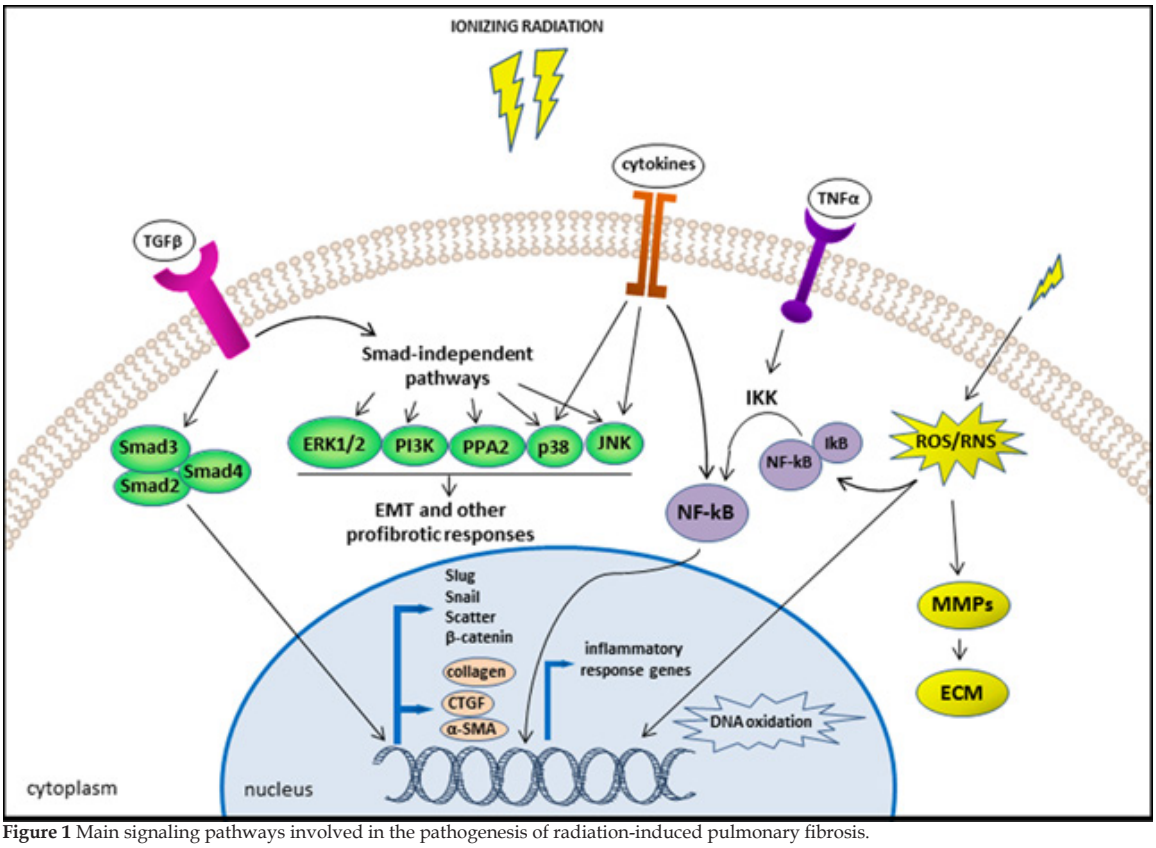

Figure 1 Main signaling pathways involved in the pathogenesis of radiation-induced pulmonary fibrosis.
α-SMA: α smooth muscle actin; CTGF: connective tissue growth factor; ECM: extracellular matrix; EMT: epithelial to mesenchymal transition; ERK1/2: extracellular-signal-regulated kinases 1/2; IkB: inhibitor of kB; IKK: IkB kinase (responsible of IkB degradation); JNK: c-Jun N-terminal kinases; MMPs: matrix metalloproteinases; NF-kB: nuclear factor-kappa-light-chain-enhancer of activated B cells; p38: p38 mitogen-activated protein kinases; PI3K: phosphatidylinositol 3-kinase; PPA2: protein phosphatase A2; RNS: reactive nitrogen species; ROS: reactive oxygen species; Smad: small mother against decapentaplegic; TGF-β: transforming growth factor β; TNFα: tumor necrosis factor α

the beneficial effects of glucocorticoids have been demonstrated in several irradiated organs and tissues[30], and Sivelestat (inhibitor of the neutrophil elastase) has been reported to decrease collagen deposition and neutrophil accumulation in damaged lungs[31].

Dividing cells, such as endothelial and epithelial cells, can also undergo a direct genetic damage, that induces cell apoptosis and a loss of integrity of pulmonary capillaries, leading to alveolar edema and finally to respiratory failure. In addition, it has been reported that various gene loci on chromosomes 1, 17, and 18 influence susceptibility to radiation-induced pulmonary fibrosis[32,33].

SIGNALING PATHWAYS INVOLVED IN RADIATION-INDUCED PULMONARY FIBROSIS

Ionizing radiation activates a quite complex biological response, mediated by several cross-talking signaling pathways and molecules[34] (Figure 1).

Cytokines and chemokines

Preclinical models showed that numerous immune cells, such as macrophages, lymphocytes, monocytes, neutrophils and basophils, are recruited into the lung after thorax irradiation[27]. At the same time, an increase in the levels of cytokines and chemokines involved in the recruitment, proliferation and activation of immune cells was observed: macrophage chemoattractant proteins (MCP)-1 and -3 and macrophage inflammatory proteins (MIPs)[35], macrophage-colony stimulating factor (M-CSF), IL-1β[27], interleukin-6 (IL-6)[36], IL-8[37], IL-17[17], CCL11 (Eotaxin)[38] and tumor necrosis factor (TNF)-α[5,39] have all been implicated in lung fibrosis. The use of anti-TNF-α antibodies or TNF-α soluble receptors, for instance, has been suggested as a potential therapeutic strategy to prevent lung fibrosis[40-42].

Among cytokines, Th2-type immune response seem to play a pivotal role in the development of pulmonary fibrosis[43,44]. The prototypical Th2 cytokine IL-4, whose levels increase during lung fibrosis[45], has been shown to exert pro-fibrotic activities, such as inducing the extracellular matrix proteins synthesis, the alternative activation of macrophages and the polarization of T cells towards the Th2 phenotype[46-48]. Another key profibrotic Th2 cytokine is IL-13[49], which promotes the differentiation of fibroblasts into myofibroblasts [50] and stimulates the transforming growth factor (TGF)-β signaling by inducing the production of latent TGF-β and enhancing the TGF-β-activating pathways[51].

TGF-β

The TGF-β system is involved in many pathological processes[52] and it has been reported to drive the majority of the cellular events associated with radiation-induced fibrosis[29,53-55].

Increases in TGF-β serum or plasma levels in patients during and after radiotherapy have been shown to be associated with a higher risk of radiation-induced lung injury[56-58]. Increased TGF-β1 and TGF-β2 concentrations have been correlated to the development of fibrosis in a murine model of radiation-induced pulmonary damage [59].

TGF-β is produced by mesenchymal, inflammatory (including lymphocytes, macrophages, eosinophils and neutrophils) and epithelial cells[60-62]. Moreover, it has been shown that certain bronchial and alveolar cells can be sources of TGF-β[63].

TGF-β is usually maintained in an inactive state by a latency-associated protein (LAP), but it is rapidly activated by a radiation-induced proteolytic cleavage, that dissociates TGF-β from LAP.

Once active, TGF-β stimulates the terminal differentiation of progenitor fibroblasts to functional fibrocytes[21,64], induces connective tissue and extracellular matrix (ECM) deposition[65], the synthesis of matrix-modifying enzymes (i.e. matrix metalloproteinases, MMPs) and stimulates the expression of α-SMA, a hallmark of myofibroblasts shown to be overexpressed in areas of active fibrosis [66]. Moreover, in the immune system, TGF-β suppresses T-cell responses[17].

The binding of TGF-β to its specific receptors TGF-βR I and II leads to the induction of the Smad family of transcriptional activators - particularly Smad3[67] - that modulate the expression of target genes and induce cellular dedifferentiation and reprogramming by interacting with specific binding sequences in the regulatory regions of the genes[29,68]. Smad proteins, in addition, can induce other transcription factors, such as Slug, Snail, Scatter, lymphoid enhancing factor-1, and beta-catenin[24], in turn facilitating EMT.

The participation of TGF-β/Smad signaling in the induction of fibrosis was demonstrated by several works, showing that Smad3-deficient animals displayed a reduced fibrotic response[20,69] and an accelerated wound healing[70] following irradiation and that a hampered Smad activity led to a significant decrease in pulmonary fibrosis development[71,72]. Indeed, antibodies against TGF-β have been reported to decrease the radiation-induced fibroblasts to fibrocytes differentiation[73] and inhibitors of TGF-βRI (SM16) or TGF-βRI serine/threonine kinase (LY2109761) have shown to reduce radiation-induced pulmonary fibrosis in animal models[74,75].

However, other studies suggested that the therapeutic suppression of the TGF-β/Smad pathway should be limited to the early phases of the disease, since this pathway appears not necessary for the maintenance of the fibrotic phenotype[76,77].

Besides the canonical Smad pathway, TGF-β can also activate non-Smad-mediated pathways, such as Rho family proteins, mitogen-activated protein kinases (MAPKs) (ERK1/2, JNK, p38), PI3K, protein phosphatase (PP) 2A and the epithelial polarity protein Par6 [78-80].

It has been reported that the extent and reversibility of EMT was influenced by the cross-talk between the classic TGF-β pathway and the above mentioned signaling molecules[5].

Accordingly, a Rho/ROCK inhibitor and statins (modulating the Smad pathway) have shown to decrease radiation-induced lung fibrosis[81].

Connective tissue growth factor (CTGF)

CTGF is induced by TGF-β, and usually acts as a mediator of TGF-β fibrotic effects[76]. However, it has been suggested that CTGF, once active, escapes regulation by TGF-β and exerts a direct profibrotic effect, contributing to the promotion and maintenance of lung fibrosis [77,82,83]. This hypothesis is supported by data demonstrating that CTGF is able to activate collagen type 1 and to exacerbate extracellular matrix synthesis[84] and that CTGF deletion in fibroblasts and smooth muscle cells greatly decreases fibrosis[85].

NF-kB signaling

The production of cytokines in immune cells is partially regulated at the transcriptional level by DNA binding proteins, such as the transcription factor NF-kB[86], which appears to be a key mediator of the cellular response to irradiation[87,88] and to play an important role in the pathogenesis of pulmonary fibrosis[89,90].

NF-kB, usually bound to the inhibitory protein IkB-α in the cytoplasm, is activated by the phosphorylation and degradation of IkB-α induced by several signals, including TNF-α[91,92], and translocates into the nucleus, where it controls the expression of a number of inflammatory response genes[93,94].

Thus, the suppression of the NF-kB network, either by protecting IkB-α from degradation or by inhibiting the NF-kB-induced up-regulation of proinflammatory and profibrogenic cytokines, could be a potential therapeutic approach for preventing or reducing lung fibrosis

ECM remodeling

The differentiation of fibroblasts to myofibroblasts during pulmonary fibrosis development lead to an increase in the synthesis of ECM proteins[95,96]. However, the changes in ECM composition, in turn, influence myofibroblast differentiation and are determining factors for the progression of the disease.

In addition to the well-known increase in vimentin and alpha-SMA expression induced by irradiation[97], the expression of elastin, type V collagen and tenascin was recently correlated to new collagen formation during lung fibrosis induction[98]. Moreover, the observations that MMP-2 and MMP-9 expression increased during radiation-induced lung injury[99] and that mice lacking MMP-13 are more resistant to pulmonary fibrosis development[100] demonstrated the role of metalloproteinases in the pathogenesis of lung fibrosis.

Surfactant Proteins

As already mentioned, when the alveolar epithelium is damaged, type II pneumocytes start proliferating to differentiate into type I cells. As a consequence, an increase in the synthesis and release of type II pneumocytes-associated surfactant proteins (SPs) in the circulation is observed[101]. In particular, the monitoring of SP-A and SP-D serum levels has been found to be associated with pulmonary fibrosis[102] and the SP-C precursor has been suggested as a marker of radiation-induced lung injury[101].

Oxidative stress

Radiation exposure leads to the production of free-radicals, mostly the reactive oxygen species (ROS, i.e. superoxide, hydrogen peroxide, and hydroxyl radical) and the reactive nitrogen species (RNS). These reactive species cause oxidative stress to the tissue [103,104] and appear to be involved in the molecular pathology of fibrosis[105] in concert with cytokines.

The radiation-induced increase in ROS/RNS levels is, indeed, associated to changes in the cytokine pattern, and leads to lipid peroxidation, DNA and protein oxidation and the activation of fibrogenic signals[106-108].

Since its participation in the pathogenesis of fibrosis, oxidative stress has been explored as a potential therapeutic intervention point. One of the most extensively studied treatment strategies are agents which act as free radical scavengers, such as thiols. In particular, the thiol amifostine has been approved by the FDA for use as a radioprotective agent[109] and is clinically used to prevent xerostomia in head and neck cancer patients undergoing radiotherapy[110].

ROS/RNS production can also be targeted by enhancing the activity of antioxidant enzymes, such as superoxide dismutases (SODs) and catalase. Several animal studies reported that the administration of a SOD transgene/liposome delivery vehicle or of SOD-catalase mimetics reduced radiation-induced pulmonary fibrosis by preventing oxidative damage[103,111,112].

Renin–angiotensin system

The renin-angiotensin system, whose main function is regulating blood pressure and fluid balance in the body, has been reported to be involved in the pathogenesis of radiation-induced lung injury[113]. Thus, this system has been proposed as a potential therapeutic target. Actually, some angiotensin-converting enzyme (ACE) inhibitors and an angiotensin receptor blocker have demonstrated success in protecting lungs from fibrosis after radiation exposure[114].

CONCLUSIONS

Radiation-induced lung fibrosis is a major radiotherapy-related toxicity, that worsens patients' quality of life and can eventually force physicians to limit the dose of cancer treatment.

Thus, intense research has been done to elucidate the molecular pathology of pulmonary fibrosis and develop therapeutic interventions that could prevent or treat this severe condition.

The development of radiation-induced pulmonary fibrosis involves multifactorial complex processes. Despite the recent progress made in understanding the molecular and cellular mechanisms underlying pathophysiology of lung injury after radiation exposure, much remains to be learned about how the various cells and signaling pathways interact.

Thus, further studies are needed to identify reliable markers and effective targets, with the aim of developing therapeutic approaches able to prevent or treat this severe condition.

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