Targeting Danger Signals to Rescue Fibrosis

Radiation therapy remains one of the main treatments for thoracic malignancies; however, its use is complicated by lung toxicity, particularly fibrosis (1). Possible alternative sources of radiation-induced injury include accidental exposure to sources of ionizing radiation. Radiation-induced pulmonary fibrosis (RIPF) may lead to long-lasting consequences that include pulmonary insufficiency, resulting in severe lifestyle alterations with progression to fibrotic lung disease and death in some individuals (1). Unfortunately, there are currently no effective therapies and/or medical countermeasures to treat induced early pneumonitis or late fibrosis. Thus, there is an unmet need to determine the molecular mechanisms underlying the profibrotic phenotype in these conditions to accelerate the development of effective therapies. In this issue of the *Journal*, Garcia and colleagues (pp. 497–509) demonstrate that extracellular nicotinamide phosphoribosyltransferase (eNAMPT), a novel damage-associated molecular pattern (DAMP) and activator of innate immune responses, contributes to the development of RIPF (2). The authors demonstrate that eNAMPT levels are elevated in animal models of RIPF and that inhibition of this protein by neutralizing polyclonal or humanized monoclonal antibodies (mAbs) or by genetic deficiency can greatly attenuate the progression of RIPF (Figure 1).

The *NAMPT* (nicotinamide phosphoribosyltransferase) gene, discovered in 1994, encodes a protein characterized as a new growth factor in the early stages of B-cell development and thus was initially named "pre–B-cell colony-enhancing factor" (3). Subsequently, the nicotinamide phosphoribosyltransferase activity of this protein and its involvement in NAD^+ (nicotinamide adenine dinucleotide) synthesis was recognized in 2001 (4). NAD^+ is a fundamental metabolite involved in energy metabolism and production (5). Genetic deletion of the *Nampt* gene in mice results in early embryonic lethality (6). Furthermore, conditional whole-body knockout of NAMPT in adult mice also leads to their death within 5–10 days. These observations underscore the importance of NAMPT in vital cellular functions such as transcription, translation, cell signaling, and metabolism (6).

NAMPT has been implicated in the pathogenesis of various disorders, including atherosclerosis, diabetes, and various types of cancer (7). In addition to the role of eNAMPT in RIPF, the same investigative group (Garcia and colleagues) has previously shown that eNAMPT, as a circulating DAMP, contributes to the development of acute lung injury and pulmonary arterial hypertension (8–10). As expected, NAMPT inhibition exerted therapeutic effects in these experimental models by reducing lung inflammation and endothelial and epithelial cell permeability (7). More recently, Garcia and colleagues have shown that early mAb-directed neutralization of eNAMPT reduced acute lung injury and inflammation in mice 4 weeks after radiation exposure (11). Thus, the results from the current study prompt further investigation into whether the antifibrotic effects of eNAMPT inhibition are mediated by reduced lung inflammation and epithelial injury or are related to direct attenuation of profibrotic responses in pulmonary fibroblasts and myofibroblasts, among the main effector cells in pulmonary fibrosis (12). Indeed, the role of inflammation in the progression of pulmonary fibrosis is complex, given that different proinflammatory cytokines and immune cells may exacerbate or ameliorate fibrotic responses (13–15). Furthermore, clinical attempts to treat pulmonary fibrosis with antiinflammatory and/or immunosuppressive agents have not been met with success, which again highlights the complexity of pulmonary fibrotic disorders (16).

To understand the mechanisms underlying the beneficial effects of eNAMPT inhibition in RIPF, Garcia and colleagues conducted an RNA sequencing analysis of irradiated lungs, which has uncovered the potential importance of an eNAMPT/Toll-like receptor 4 (TLR4) signaling pathway in the pathogenesis of RIPF. In parallel, Li and colleagues have recently shown that citrullinated vimentin can serve as a ligand for TLR4 and activate profibrotic responses in lung fibroblasts (17). Another endogenous ligand for TLR4, the high mobility group box protein 1, has been shown to activate myofibroblast transformation in lung fibroblasts by activating NF-κB–mediated expression of TGF-β1 (transforming growth factor-β1) (18). Thus, in addition to a known role of eNAMPT as a proinflammatory cytokine, eNAMPT may directly affect profibrotic responses in lung fibroblasts through interactions with TLR4.

In addition, Garcia and colleagues show that anti-NAMPT neutralizing mAb treatments normalized TGF-β1/Smad and TLR4 signaling in RIPF as well as reversed the expression of a number of other differentially expressed genes potentially related to RIPF pathogenesis, including profibrotic regulators and microRNAs. Further, the mAb therapy restored redox homeostasis by downregulating the expression of NOX4 (NADPH oxidase-4) expression, a source of reactive oxygen species, and increasing Nrf2 expression, a master regulator of the antioxidant response.

In the present study, the investigators show that eNAMPT concentrations are increased in nonhuman primates after radiation exposure. Preclinical studies of eNAMPT neutralization in larger animals are needed to determine safety and efficacy and will represent an important step to support the clinical application of eNAMPT neutralization in humans.

In summary, the article by Garcia and colleagues highlights the pathophysiological importance of eNAMPT in RIPF and warrants future investigation of the underlying mechanisms (Figure 1). These findings support the notion that intracellular NAMPT, which regulates fundamental metabolic processes in homeostasis, can be released as a DAMP into the extracellular milieu under stress conditions and propagate cellular responses to injury (7). Further identification and characterization of eNAMPT and other DAMPs or circulating factors released in fibrotic conditions will be critical as

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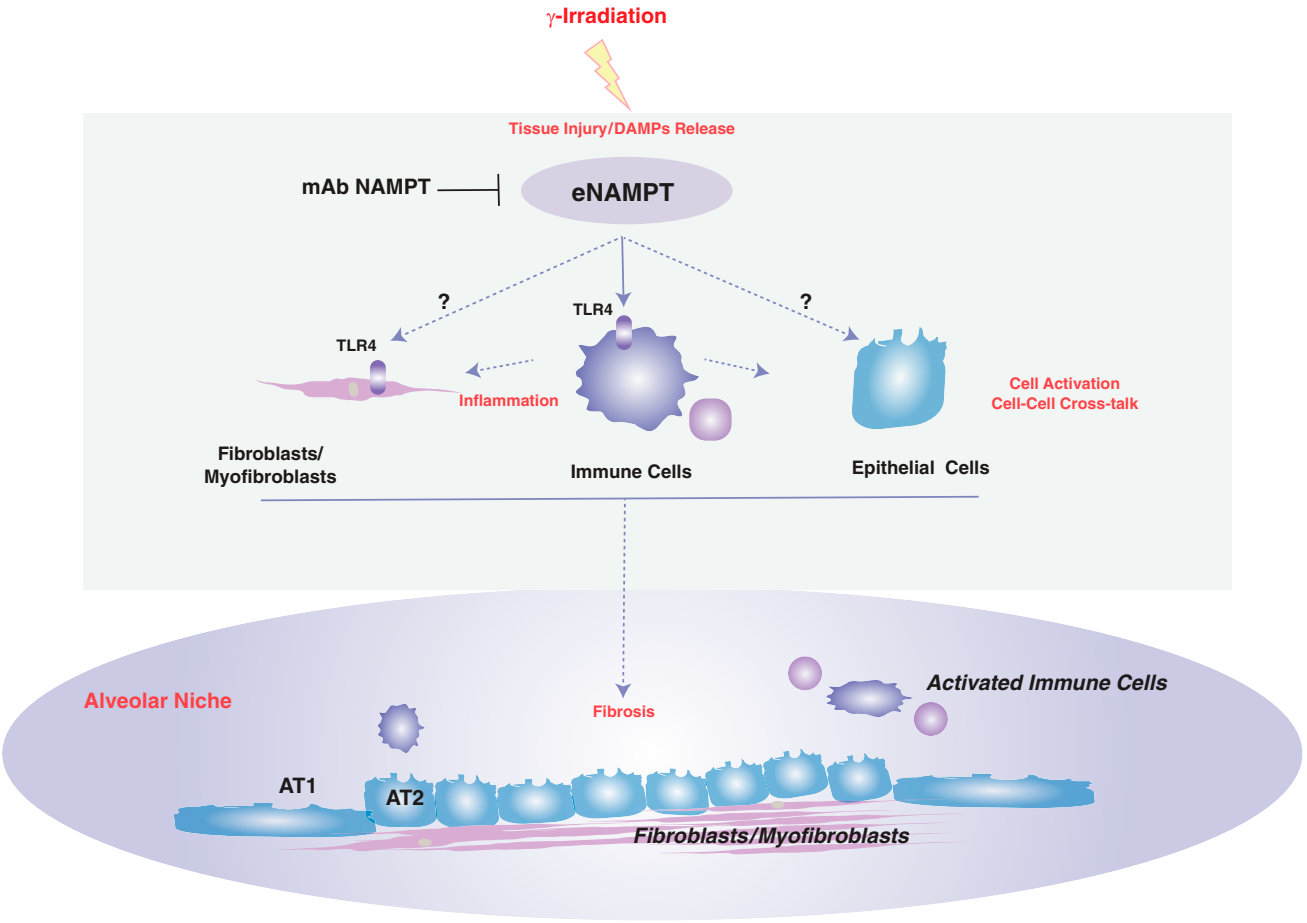

Figure 1. Schematic overview of how eNAMPT may contribute to radiation-induced lung injury and fibrosis. Exposure to γ-radiation, an experimental model of pulmonary fibrosis, can cause tissue injury associated with release of DAMPs. eNAMPT is a novel DAMP that can aggravate proinflammatory responses. Garcia and colleagues show that eNAMPT release contributes to radiation-induced pulmonary fibrosis (RIPF) in mice. The release of eNAMPT can activate inflammatory cells within the profibrotic niche via activation of TLR4. eNAMPT may also potentially activate fibroblasts/myofibroblasts indirectly via macrophage-dependent inflammation or also directly via TLR4 activation. Direct or indirect activation via cross-talk of other pulmonary cell types by eNAMPT, such as AT1 or AT2, warrants further investigation. Collectively activation of inflammatory cells, fibroblasts/myofibroblasts and other cell types within the alveolar niche may promote profibrotic responses. The studies of Garcia and colleagues further show that blocking eNAMPT can reduce tissue injury and fibrosis in models of RIPF, implicating eNAMPT as an attractive therapeutic target in IPF and other fibrotic lung diseases. AT1, AT2 = Type I or Type II epithelial cells; eNAMPT = extracellular nicotinamide phosphoribosyltransferase; mAb = monoclonal antibodies; RIPF = radiation-induced pulmonary fibrosis; TRL4 = Toll-like receptor-4.

they may serve as targets for therapeutic intervention to treat pulmonary fibrotic disorders. ■

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