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## Mechanisms of Normal Tissue Injury from Irradiation

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### Abstract

Normal tissue injury from irradiation is an unfortunate consequence of radiotherapy. Technologic improvements have reduced the risk of normal tissue injury, however toxicity causing treatment breaks or long term side effects continue to occur in a subset of patients. The molecular events that lead to normal tissue injury are complex and span a variety of biologic processes, including oxidative stress, inflammation, depletion of injured cells, senescence, and elaboration of pro-inflammatory and pro-fibrogenic cytokines. This manuscript describes selected recent advances in normal tissue radiobiology.

### Introduction

Normal tissue injury from irradiation is a key consideration for treatment of any condition with radiotherapy. The doses chosen for therapy are generally a compromise between the dose that effectively sterilizes cancer in the majority of patients and the dose that causes complications in the smallest subset. Although highly conformal therapies such as intensity modulated radiation therapy (IMRT) and stereotactic body radiation therapy combined with image guided treatments have made substantial progress in reducing the exposure of normal tissues to the prescription dose, toxicity continues to occur, albeit at a lower frequency. Understanding the molecular events responsible for the perpetuation of normal tissue injury is critical to developing methods to prevent, mitigate, and treat these toxicities.

Ionizing radiation results in the immediate generation of highly reactive free radicals with resulting rapid protein modifications and damage to DNA, RNA, and cell membranes<sup>1</sup>. Although a substantial amount of this damage may be repaired over time, it is increasingly recognized that these early events can stimulate changes in cellular function and inflammation that can persist over months to years<sup>2</sup>. Recent discoveries about the molecular events that drive normal tissue injury and the appreciation of the importance of tumor in this response have complemented the traditional radiobiological understanding of normal tissue

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injury. As it is impossible to review all of the potential mechanisms of radiation injury, this manuscript will summarize some of the recent advances in this context and highlight potential areas of opportunity for further preclinical and clinical exploration.

## Oxidative Stress

Oxidative stress has long been thought to contribute to both acute and chronic radiation injury. Following the primary events of radiation exposure (hydrolysis of water and direct ionization), secondary reactions result in chronic elevation of reactive oxygen species (ROS), which are formed and propagated for extended periods of time<sup>3</sup>. In addition, ROS can be generated by a host of cell types in the setting of injury and inflammation, which can contribute to ongoing oxidative injury through generation of reactive nitrogen species (RNS)<sup>4</sup>. For example, injured endothelial cells, epithelial cells, and inflammatory cells can produce compounds such as superoxide and nitric oxide<sup>5, 6</sup>, which may further perpetuate local oxidative stress, resulting in ongoing injury after radiation exposure. Many attempts at minimizing injury from irradiation have focused on reducing oxidative stress by inhibiting the production of free radicals and delivering compounds capable of scavenging deleterious free radicals. Collectively, these studies have contributed greatly to our understanding of the mechanisms of normal tissue injury from irradiation.

One such radical, superoxide, is extremely toxic and is generated by NADPH oxidase in the process of normal metabolism, in the setting of immune response to pathogens, and as a consequence of injury, such as irradiation<sup>7</sup>. Superoxide can react directly with DNA or other cellular components to induce damage or can generate other harmful secondary radical species<sup>8</sup>. A large literature exists that has demonstrated delivery to or elaboration of superoxide dismutase (SOD), a detoxifying enzyme, in irradiated cells or tissues can reduce the deleterious effects of irradiation. Indeed, delivery of SOD and SOD modified to enhance delivery and stability have been successful in reducing the toxicity of radiation in clinical trials<sup>9, 10</sup>. Unfortunately, some studies have also failed to show a benefit and have reported allergic reactions<sup>11</sup>. Based on extensive preclinical data supporting SOD gene therapy as a potential therapeutic to prevent radiation injury<sup>12</sup>, alternative methods of SOD delivery, such as an SOD plasmid liposome, have successfully been delivered in Phase I trials<sup>13</sup>. The difficulty in effective and efficient delivery of SOD to irradiated tissue has spurred the development of alternative agents that can act similarly, also known as SOD mimetics. One concern with this approach is the possibility of tumor protection if the agent is not highly selective to normal tissue compared to tumor.

Indeed, delivery of agents capable of scavenging superoxide and ROS, such as genistein, AEOL 10150, EUK-189, and EUK-207, are capable of reducing tissue markers of oxidative stress and minimizing inflammation and radiation injury<sup>14–20</sup>. In general, these scavenging agents tend to reduce not only markers of oxidative stress in irradiated tissue, they also reduce inflammatory cell infiltration and pro-inflammatory, pro-fibrotic, and immunomodulatory cytokine expression<sup>14</sup>. In some cases, these global anti-inflammatory effects are capable of reducing what appears to be secondary injury, consistent with the hypothesis that ongoing inflammation can perpetuate continued injury in irradiated tissue<sup>14</sup>.

Although the delivery of mimetics is a promising strategy, the identification of redox sensors which can activate transcription of a wide range of genes important in the cellular response to oxidative stress has opened new potential opportunities for understanding radiation normal tissue injury and mitigating the deleterious effects of radiation. One such pathway is the Nrf2-ARE pathway. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of at least 200 genes, many of which are oxidative stress related proteins, including NADPH quinone oxidoreductase 1<sup>21</sup>, hemoxygenase-1 (HO-1), SOD, and glutathione S-transferase proteins<sup>22-25</sup>. Loss or constitutive expression of Nrf2 can both be deleterious, suggesting that expression of Nrf2 must be balanced appropriately to the to the physiologic state<sup>26, 27</sup>.

Keap1 is a protein that binds to Nrf2 and facilitates its ubiquitination and proteolysis<sup>28</sup>. Interestingly, mutations in Keap1 and Nrf2 are frequently found in cancers and are associated with local recurrence after radiation and cellular radiation resistance<sup>29, 30</sup>. In the setting of radiation, NADPH oxidase activity is increased in lung tissue<sup>6</sup>, which is known to enhance, and in some settings be required for, Nrf2 expression<sup>31, 32</sup>, presumably reducing lung injury. Studies in Nrf2 deficient mice have demonstrated a substantial increase in late lung injury after localized irradiation<sup>33, 34</sup>. This injury is associated with an enhanced level of isoleuvoglandin (IsoLG) modification of proteins, an event that can alter protein function<sup>35</sup>. Interestingly, radiation also reduces Nrf2 expression in lung<sup>36</sup>, potentially further increasing the amount of IsoLG adducts. Recently published work has demonstrated that this enhanced IsoLG modification can result in enhanced apoptosis of pulmonary cells and can render collagen resistant to MMP-1 mediated degradation<sup>35</sup>. These findings not only provide a novel potential biomarker of injury, but also fundamentally alter our understanding of how redox sensitive pathways can cause perpetuation of injury long after the radiation exposure. Further, they provide an interesting rationale for the challenge in reversing established chronic radiation injury.

## Metabolism

Metabolism is increasingly recognized as an important aspect of tissue homeostasis, which can be altered in the setting of malignancy. In general, tumor cells are metabolically heterogeneous and often times adaptable in their capacity to utilize various substrates as energy and carbon sources<sup>37</sup>, often utilizing anaerobic metabolism preferentially. Recently, it has become increasingly apparent that diseased tissue may also have metabolic derangements, partially due to hypoxia, inflammation, and the resulting activation of numerous pathways as a result that may also impact metabolism, such as HIF-1 $\alpha$  and mTOR<sup>38</sup>. Indeed, hypoxia and oxidative stress in injured normal tissue after irradiation corresponds to enhanced HIF-1 $\alpha$  signaling, which in turn parallels the induction of other known drivers of injury, such as TGF $\beta$ 1 and VEGF<sup>39, 40</sup>. The presence of hypoxia in irradiated tissues has been confirmed in studies of irradiated human tissue that demonstrated morphologic evidence of radiation injury<sup>41</sup>.

Importantly, metabolism cannot be entirely separated from oxidative stress and hypoxia, as these three processes contribute to each other in many ways. For example, assessment of tetrahydrobiopterin (BH4), a non-enzymatic redox metabolite, has revealed reduced

availability of BH4 after potentially damaging radiation exposures<sup>42</sup>. BH4 deficiency is thought to contribute to higher superoxide generation due to lack of BH4 complex formation with endothelial nitric oxide synthase<sup>43</sup>. As a result, tissue is subjected to enhanced oxidative stress and secondary formation of peroxynitrite<sup>44</sup>. This work demonstrates that metabolites may provide a window for mechanistic understanding of alterations in normal tissue and a potential marker of injury<sup>45</sup>.

The development of novel non-invasive methods to assess metabolism in living organisms has provided evidence that radiation can alter the metabolism of normal tissues. Magnetic resonance spectroscopy of hyperpolarized <sup>13</sup>C pyruvate allows the non-invasive imaging of the fermentation of pyruvate to lactate. Using this approach, Thind et al<sup>46</sup> demonstrated increased conversion of pyruvate to lactate in lung tissue irradiated to a fibrosis evoking dose compared to adjacent unirradiated normal tissue, consistent with altered metabolism as a consequence of injury.

Although metabolism plays a critical role in cancer and injury, it will be challenging to dissect the impact of metabolism when agents with multiple targets, including metabolism, are used to modify injury after irradiation. For example, mTOR, Akt, and other main signal transduction hubs play a large role in altering metabolism, but they may also alter a tremendous number of biological processes, presumably independent of metabolism. Assessment of specific inhibitors of metabolism currently being developed for cancer therapy in this context may provide interesting biologic information about metabolism in radiation injury and unique challenges and opportunities for intervention.

### **TGF- $\beta$ and TNF- $\alpha$ signaling in normal tissue toxicity**

TGF- $\beta$  is a cytokine with a wide array of functions that is generally secreted by cells in a latent form. Activation of the latent peptide occurs after various stimuli, including ROS exposure<sup>47</sup> and the actions of proteinases<sup>48</sup>, results in liberation of the active TGF- $\beta$  protein and binding to one of several TGF- $\beta$  receptors. Signaling through the TGF- $\beta$ 1 receptor is perpetuated by the Smad family of signaling proteins and transcription factors, eventually resulting in transcription and repression of an array of genes involved in cell proliferation, epithelial-mesenchymal transition, immune suppression, and inflammation<sup>49</sup>. Additional Smad independent TGF- $\beta$  signaling pathways also exist, such as signaling via the RHO, ERK, JNK, and p38 MAP pathway<sup>49</sup>.

Radiation induces a dose and time dependent increase in TGF- $\beta$  activity in tissue, with increased TGF- $\beta$  expression detected within minutes to hours after irradiation<sup>50</sup>. Although TGF- $\beta$  expression can return to baseline after this initial increase, it often rises again in irradiated tissue in the setting of chronic injury<sup>50</sup>. TGF- $\beta$  is known to play a central role in a number of pathologic states characterized by tissue remodeling and inflammation, such as radiation injury. Indeed, data from human studies have suggested that plasma TGF- $\beta$  is increased in settings associated with tissue injury, such as after thoracic radiotherapy. In this situation, plasma TGF $\beta$  concentrations may identify patients at low risk of radiation pneumonitis and late lung injury<sup>51-55</sup>, although controversy exists about whether an increase in TGF- $\beta$  concentrations is an independent predictor of risk of lung injury<sup>56</sup>. Regardless,

these data, a wealth of preclinical data, and other clinical series strongly support the importance of TGF- $\beta$  in clinical radiation injury.

Agents targeting TGF- $\beta$  signaling via targeting the TGF- $\beta$ 1 receptor or downstream intermediates have shown efficacy in reducing both acute and chronic radiation injury in preclinical models<sup>57-60</sup>. For late injury, the greatest degree of mitigation was observed with chronic dosing beginning immediately after exposure<sup>57</sup>. Similarly, delivery of halofuginone, a compound capable of inhibiting TGF- $\beta$ 1 receptor signaling, is capable of preventing radiation induced fibrosis of skin<sup>61</sup> and lung<sup>62</sup> while simultaneously enhancing tumor response to irradiation<sup>63</sup>. Although preclinical studies of TGF- $\beta$  signaling inhibition have been promising, clinical translation in non-oncologic settings has been limited by concern for toxicity with chronic systemic administration of these inhibitors. These concerns are based on observation of inflammation, autoimmunity, and developmental defects in knockout mice<sup>64, 65</sup> and additional toxicities observed in preclinical studies<sup>66, 67</sup>. Although these effects have not been seen in clinical trials of such agents, development of reversible cutaneous squamous cell carcinomas and keratoacanthomas has been observed<sup>68</sup>. The eventual importance of these agents in managing radiation injury remains uncertain given that available preclinical data suggests chronic dosing may be necessary for optimal effect.

Tumor necrosis factor alpha (TNF- $\alpha$ ) is an inflammatory cytokine involved in the acute phase reaction, that is rapidly and persistently expressed in irradiated and adjacent tissue<sup>69</sup>. The delivery of antioxidants can, in some cases, prevent TNF- $\alpha$  elaboration and reduce radiation injury<sup>70</sup>. Deficiency of TNF- $\alpha$  in a lung injury model was sufficient to prevent symptoms of radiation pneumonitis<sup>70</sup>. TNF- $\alpha$  has also been implicated in radiation mucositis, enteritis, and dermatitis<sup>71-75</sup>. The clinical availability of agents targeting TNF- $\alpha$  to treat rheumatologic disorders provides a unique opportunity for clinical translation, and will likely enhance investigation into this molecule and its pathway.

## Senescence in Radiation Injury

Another recent area of intense interest in radiation normal tissue injury is senescence of normal tissue stem cells. Senescence is a state of permanent growth arrest that occurs in several contexts, including as a normal part of development and aging. Replicative senescence occurs as the telomeres of dividing cells reach a limiting length after successive division, preventing further replication. In contrast, stress induced cellular senescence occurs after exposure to oxidative stress or DNA damaging stimuli, such as radiation. Recent evidence supports the concept that senescence plays an important role in radiation-induced normal tissue injury<sup>6, 76</sup>. Although many cell types appear to be susceptible to senescence after irradiation, the senescence that occurs in normal tissue stem cells may be particularly harmful due to the inability of the senescent stem cells to replace lethally damaged parenchymal cells after irradiation<sup>6, 76</sup>.

Although parenchymal depletion due to reduced regenerative capacity clearly plays an important role in the deleterious effects of senescence in normal tissue after irradiation, senescent cells are not merely inert bystanders<sup>6</sup>. A complex mixture of pro-inflammatory/immunomodulatory, pro-angiogenic, and mitogenic molecules are secreted by senescent

cells, commonly referred to as the secretory profile of senescence (SASP)<sup>77</sup>. Importantly, several molecules of the SASP have been associated with normal tissue injury after irradiation, including IL-1, IL-6, TGF $\beta$ , EGF, VEGF, and TNF- $\alpha$ . Thus, senescent cells may further enhance or perpetuate normal tissue injury through the elaboration of these molecules with resulting paracrine effects such as inflammation, tissue remodeling, and secondary senescence and cell death.

Senescence of normal tissue stem cells may play a role in both the acute and late normal tissue responses to irradiation and have been identified in the intestine, skin, lung, kidney, bone marrow, mucosa, and many other organs<sup>76, 78, 79</sup>. At an organism level, accelerated aging and frailty has been appreciated as a consequence of cytotoxic therapies, such as irradiation, in preclinical studies and in patients<sup>76, 80–82</sup>. Although a number of agents have been identified that are capable of preventing senescence and preventing normal tissue injury, particularly in the setting of fibrosis<sup>6, 83</sup>, there are relatively few studies demonstrating that clearance of established senescence can reverse injury (treatment) or ameliorate progression (mitigation)<sup>76</sup>.

## Macrophage Polarization and Radiation Injury

The field of immunology has developed at an explosive pace over recent years, and a growing appreciation of the immunologic effects of irradiation has been driven by the use of irradiation as an immune adjuvant in the context of cancer immunotherapy. Large volume irradiation has the greatest capacity for immunosuppression due to marrow suppression and a reduction of circulating or resident immune cells. In contrast, localized irradiation can induce a sterile inflammation (non-infectious) that is chronic in nature and can result in both acute and late toxicities.

Investigations of immunologic phenomenon and their contribution to normal tissue toxicity have focused on many organs, but perhaps most thoroughly in models of lung injury. For many years, it has been known that radiation drives an inflammatory response in the lung which can remain asymptomatic or progress to radiation pneumonitis and eventual radiation fibrosis. Indeed, radiation injury of the lung is generally treated with immunosuppressive glucocorticoids<sup>84</sup>. Although it is accepted that radiation lung injury is characterized by inflammatory infiltration, a more complete understanding of the complexities of the post-irradiation inflammatory state has been accumulating as interest in the intersection of radiation and immunologic phenomenon has expanded exponentially.

One of the major components of the accumulated inflammatory infiltration after irradiation in lung and other organs are macrophages<sup>85–88</sup>. Macrophages are known to play a key role in wound healing and repair, however they may also contribute to pathologic responses such as fibrosis<sup>89</sup>. Macrophages exhibit substantial plasticity, with the capacity to polarize into a state of activation and alternative activation depending on the surrounding microenvironment<sup>90</sup>, with resulting phenotypic and functional changes. The polarization of macrophages exists along a spectrum, with varying nomenclatures, however frequently is defined as classically activated (M1) and alternatively activated (M2). Alternatively activated macrophages, also known as regulatory or suppressive macrophages, can be generated after

exposure to type 2 cytokines, such as IL-4 and IL-13. These macrophages can secrete IL-10 and act in an immunoregulatory, and in some cases, suppressive fashion. An additional subset of macrophages, termed profibrotic macrophages, that have been exposed to IL-4 and IL-13 secrete TGF $\beta$  and mitogenic factors<sup>89</sup>.

The relative numbers of phenotypically distinct macrophage subsets vary between fibrosis prone and fibrosis resistant strains of mice after irradiation<sup>86</sup>, with a notable accumulation of macrophages expressing markers of alternative activation, implicating type 2 inflammation as a driver of acute and late radiation lung injury. Studies of mice deficient in IL-4, a type 2 cytokine that is associated with alternatively activated macrophages, has suggested that the development of fibrosis observed after radiation is not dependent on IL-4, however the recovery of macrophage subpopulations and the phenotypes of accumulated macrophages can be substantially altered after irradiation in the absence of IL-4<sup>87</sup>. In contrast, deficiency of IL13 through genetic manipulation or through systemic delivery of a therapeutic neutralizing antibody is sufficient to substantially reduce radiation fibrosis and the accumulation of alternatively activated macrophages in mouse models<sup>85</sup>. IL-13 deficiency reduces TGF $\beta$  activity and the expression of numerous downstream fibrosis associated genes in irradiated tissue<sup>85</sup>. It is not yet known if combined deficiency of IL-4 and IL-13 will further reduce the pneumonic changes after irradiation and further suppress late lung injury. Regardless, a number of agents targeting IL-13 are in late stage clinical trials for the treatment of asthma, autoimmune disorders, and allergic disease<sup>91</sup>, which may provide an opportunity for clinical translation in the setting of radiation injury once the optimal timing of delivery can be clarified.

Recently, additional pro-inflammatory feedback mechanisms, such as enhanced IFN- $\gamma$  dependent inflammation, have been identified after suppression of IL-13 in fibrosis models, lending support to the notion that combined targeting of multiple pathways may be a critical component of effective therapy<sup>92</sup>. Further study of the role of type 2 inflammation on both the acute and late effects of irradiation is likely to clarify the extent to which agents and combination of agents targeting these processes may provide therapeutic opportunities. Because type 2 immunity has a known role in driving chronic inflammation in a number of pathologic conditions, such as asthma and idiopathic pulmonary fibrosis, drug development in this area is expanding, providing a tremendous opportunity for eventual clinical translation<sup>93</sup>.

## T cells as a driver of radiation injury

Although T-cells do not account for a large proportion of the accumulated cells in irradiated tissues, they are capable of dramatically altering immune responses through effector mechanisms, and are known to drive chronic inflammatory states such as graft versus host disease and other automimmune phenomenon, resulting in pathologies strikingly similar to those observed after irradiation<sup>94</sup>. Indeed, T cell polarization and altered balance in T cell subsets has been implicated as a possible contributor to radiation injury<sup>94</sup>. Subsets of T lymphocytes are known to have differential sensitivities and recovery rates after irradiation, potentially leading to an altered balance of T cell subsets after irradiation<sup>95-98</sup>. Perhaps most

importantly, selective depletion of T cell subsets has provided evidence that T cells can drive some aspects of radiation injury<sup>99-101</sup>.

Clinical data has suggested that the presence of CD4+ T cells have a higher risk of developing pneumonitis<sup>102</sup>, although from these studies it is not clear if CD4+ cells are a marker of radiation lung injury or drive the phenomenon. It is important to consider that CD4+ T helper (Th) cells can be further differentiated into several lineages, including the Th1, Th2, and Th17 lineage. Irradiation shifts inflammation towards a T helper 2 (Th2) dominant response and away from a Th1 response<sup>103</sup>, potentially activating damaging chronic inflammatory processes. Similarly, Th17 subsets and Treg subsets have been demonstrated to infiltrate irradiated lung tissue<sup>104</sup>. The importance of Th17 subsets in radiation injury is supported by the capacity of IL-17 targeted therapies to reduce the severity of radiation injury in animal models<sup>105</sup>.

Conceptually, these data are consistent with the above importance of type 2 inflammation, as IL-17 has been implicated in contributing to type 2 immune responses<sup>106</sup>. In contrast, depletion of Treg enhance Th17 responses, and mitigate lung injury after irradiation<sup>101</sup>. Clearly, given the complexity of Th17 responses, including the potential for a regulatory or pro-inflammatory component, there is still much to learn in terms of these immune subsets that may help modify toxicity of radiotherapy.

## FLASH

Although dose rate has long been known to alter tumor and normal tissue responses to irradiation, some recent findings have fundamentally altered the concept of normal tissue effects from radiation. Studies of ultra-high dose rate irradiation (>40 Gy/second) delivered in short pulses, also known as FLASH irradiation, have suggested a remarkable tumor selectivity of cell killing<sup>107</sup>. The use of similar doses of FLASH and conventional irradiation (<0.03Gy/second) resulted in similar tumor responses. In contrast, normal lung tissue irradiated with FLASH irradiation did not demonstrate the typical rapid onset of apoptosis and TGF $\beta$  activation that was seen with conventional delivery. Further, mice treated with FLASH at doses as high as 17 Gy to the thorax did not develop pulmonary fibrosis. Although fibrosis was achieved in an attenuated form by giving 30 Gy whole thorax FLASH, this dose is rapidly fatal using conventional dosing methods.

The exact mechanism for the reduction in normal tissue injury using the FLASH approach is continuing to be studied. A reduction in apoptosis in multiple normal tissue cell types immediately after exposure with FLASH compared to the same conventional dose was appreciated<sup>107</sup>. Although delivery of TNF $\alpha$  prior to FLASH was capable of massively increasing apoptosis and pulmonary edema, it was not capable of inducing fibrosis, suggesting that normal tissue apoptotic sparing is not the primary mechanism of tumor selectivity of FLASH.

The ability to study and implement the FLASH phenomenon has been limited by the technical capacity to deliver such rapid dose pulses. Recently, a method for LINAC based FLASH was described, potentially allowing more widespread testing of the methods<sup>108</sup>.



This has allowed confirmation of a normal tissue sparing effect of FLASH in an intestinal injury model<sup>109</sup>. Additional studies are anticipated to evaluate selectivity of FLASH in other tissues and to further understand the mechanism of preferential normal tissue sparing.

## Conclusions

Radiation normal tissue injury is a complex process that spans acute and late injury in biologically diverse tissues. Classical radiobiological understanding of normal tissue injury has provided a framework into which molecular biologic, immunologic, and tissue homeostatic understanding is now being integrated. This enhanced understanding of radiation injury provides new possibilities for mitigation and therapeutic intervention. The development of novel assays and tools coupled with an exponentially increasing knowledge of the effects of radiation on the immune system suggests that numerous additional advances are on the horizon.

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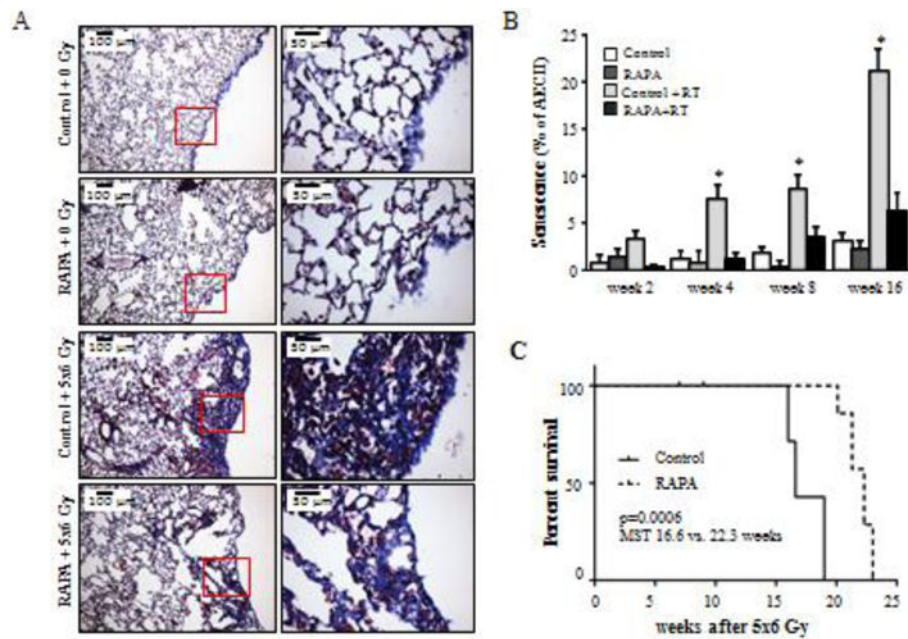
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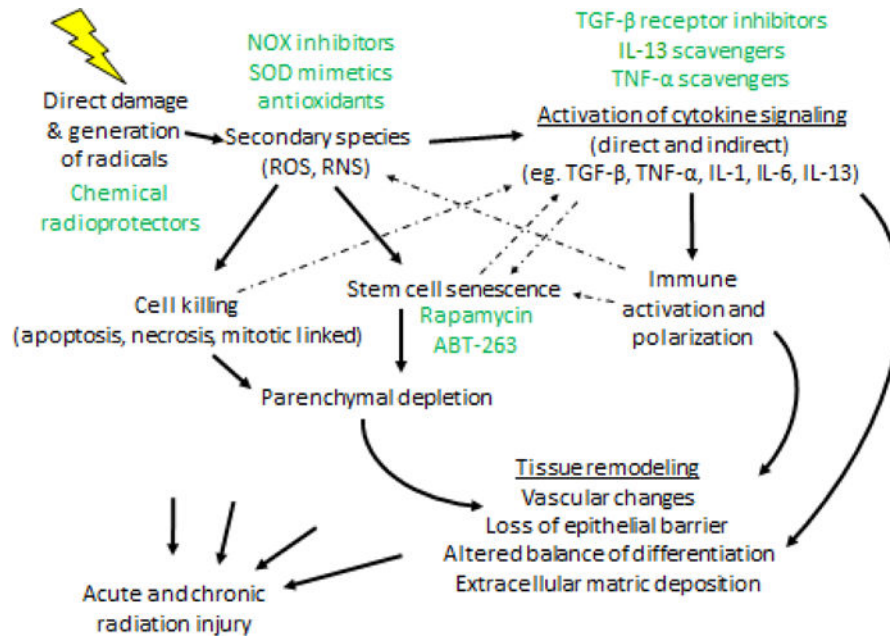
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### Figure 1. Senescence in radiation injury

C57BL/6NCr Mice were exposed to 5×6 Gy of thoracic IR and treated with rapamycin or control diet. A) Masson trichrome staining of lung tissue at 16 weeks after IR. Collagen: blue, nuclei: purple, cytoplasm/epithelia: pink. B) The percentage of type II pneumocyte (AECII) cells staining for  $\beta$ -Gal activity at 2, 4, 8, and 16 weeks after IR was scored. A) Representative images at week 16 after IR. Percentage of AECII co-stained for Pro-surfactant-C (Pro-SP-C) at 16 weeks after IR. C) Kaplan-Meier survival analysis demonstrated that administration of rapamycin extended survival compared to mice receiving control diet after IR. Columns: mean, error bars: SD, brackets:  $p < 0.05$  by ANOVA. Reproduced with permission from<sup>110</sup>.



**Figure 2. Pathways of radiation injury**

Radiation induces direct damage in normal tissues but also can generate free radicals, which can themselves cause injury or give rise to secondary species that may also cause injury. As a consequence, normal tissue cells are killed through a variety of mechanisms. Normal tissue stem cells may enter a state of senescence, which when combined with the initial cell killing, may result in parenchymal depletion and resulting tissue dysfunction. Simultaneously, ROS can initiate cytokine signaling, as can cell death and senescence. Collectively, these effects lead to immune activation and alterations in immune cell polarization, that can lead to chronic inflammation and further oxidative stress. Potential prevention, mitigation, and treatment approaches are highlighted in green.