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RECENT PROGRESS IN DEFINING MECHANISMS AND POTENTIAL TARGETS FOR PREVENTION OF NORMAL TISSUE INJURY AFTER RADIATION THERAPY

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The ability to optimize treatments for cancer on the basis of relative risks for normal tissue injury has important implications in oncology, because higher doses of radiation might, in some diseases, improve both local control and survival. To achieve this goal, a thorough understanding of the molecular mechanisms responsible for radiation-induced toxicity will be essential. Recent research has demonstrated that ionizing radiation triggers a series of genetic and molecular events, which might lead to chronic persistent alterations in the microenvironment and an aberrant wound-healing response. Disrupted epithelial–stromal cell communication might also be important. With the application of a better understanding of fundamental biology to clinical practice, new approaches to treating and preventing normal tissue injury can focus on correcting these disturbed molecular processes. © 2005 Elsevier Inc.

Radiation therapy, Complications, Cytokines, Treatment.

INTRODUCTION

The tolerance of normal tissues continues to be an impediment to the optimal use of radiation therapy in the treatment of cancer (reviewed in Stone *et al.* [1]). Much progress has been achieved recently toward better elucidating the molecular mechanisms underlying radiation injury (1–8). This work has resulted in the exploration of more targeted therapies designed to protect normal tissues from radiation damage (9, 10). Herein, some of these recent mechanistic findings will be highlighted, as will potential approaches to the treatment and prevention of radiation injury.

MOLECULAR BIOLOGIC ADVANCES

Late complications of cancer therapy are becoming an increasingly important concern to both physicians and patients as the number of long-term cancer survivors increases. For the radiation oncologist, a better understanding of the molecular events underlying normal tissue injury will permit a more rational approach to the prevention and treatment of normal tissue injury (11). Currently, the physician must try to prevent complications primarily through restricting the dose and volume to be irradiated (12). The relationship between dose, volume, complications, and tumor control is complex and is not precisely defined for most cancers and normal tissues (12, 13). Only recently have investigators attempted to better delineate these relationships by taking advantage of innovations in radiation dose delivery and imaging technology.

Over the past several years, major advances in the tools of molecular biology have enabled scientists to move rapidly toward a better understanding of underlying mechanisms responsible for radiation-induced normal tissue injury. We have known for decades that the biologic effects of ionizing radiation begin with the generation of reactive oxygen species (ROS) (14). More recently, we have learned how these immediate biochemical events rapidly trigger a series of genetic and molecular phenomena leading to clinically and histologically recognizable injury (4, 5, 15–24). This process is dynamic and involves a number of proinflammatory cytokines, profibrotic cytokines, and chemokines produced by macrophages, epithelial cells, and fibroblasts. Furthermore, these events seem to be sustained for months or years beyond the completion of therapy (6); however, the mechanisms responsible for maintaining the injured phenotype, until recently, have remained unknown (17, 25).

On the basis of knowledge regarding how radiation in-

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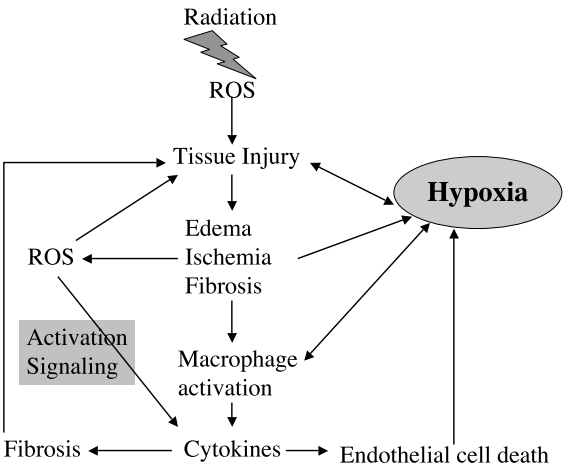

Fig. 1. A new paradigm for hypoxia-mediated chronic injury. The initial damage from irradiation is initially generated by the direct action of reactive oxygen species (ROS) on DNA. This results in tissue injury, including endothelial cell damage, with an increase in permeability, edema, and fibrin accumulation in the extracellular matrix. Subsequently, an inflammatory response ensues, including macrophage accumulation and activation. The macrophages release a number of cytokines and ROS. Both vascular changes, leading to decreased oxygen delivery, and increased oxygen consumption, due to macrophage activation, contribute to the development of hypoxia. Hypoxia further stimulates production of ROS and profibrogenic and proangiogenic cytokines. This perpetuates tissue damage, leading to fibrosis via transforming growth factor β (TGFβ) production/activation and stimulates angiogenesis through vascular endothelial growth factor (VEGF) production. In an attempt to respond to the proliferative stimulus of VEGF, endothelial cells are dying due to previously accumulated radiation damage. Hypoxia, therefore, perpetuates a nonhealing tissue response, leading to chronic radiation injury.

teracts with tissue to create free radicals, numerous biochemical compounds, most notably the thiols, have been administered to target oxygen and oxygen-free radicals in an attempt to reduce radiation-induced injury (26). Most studies have limited delivery of these agents to the period of radiation treatment, under the assumption that this is the time at which the highest concentration of ROS will be present (26). Recent data, however, suggest that ROS and/or their byproducts are continuously overproduced after the initial injury and thus might be, in part, responsible for late radiation-induced damage. For example, Vujaskovic *et al.* (17) have noted the presence of progressive normal tissue hypoxia after pulmonary irradiation. Because hypoxia itself is known to generate ROS, promote inflammation and vascular permeability, activate the profibrotic cytokine transforming growth factor β (TGFβ), and promote collagen formation (27–29), these investigators argued that postradiation hypoxia might be an important contributor to the maintenance of the injured phenotype. This finding suggests a new paradigm for normal tissue injury, which is currently under investigation (Fig. 1). In many ways, irradiated tissue responds to its injury in a manner very similar to normal wound healing. In the case of irradiated tissues, however, the wound does not heal normally but instead enters into a "death spiral" containing features of hypoxia, angiogenesis, cell death, proliferation, and macrophage infiltration. Ultimately, this spiral leads to total replacement of the tissue by collagen, leaving few, if any, cellular elements.

NEW APPROACHES TO PREVENTION AND TREATMENT

This new information about the pathogenesis of radiation-induced normal tissue injury has created opportunities to identify potential molecular targets for treatment or prevention of side effects. Among the most promising avenues for intervention is the TGFβ pathway. This cytokine has been demonstrated to be a key mediator of fibrogenesis in a number of pathologic conditions, including after irradiation (30). It has also been suggested as a therapeutic target in fibrosing renal diseases (31, 32). Irradiation results in increased expression and activation of TGFβ, which promotes the deposition of fibrous tissue and inhibits epithelial repair (30, 33). These changes in the bioavailability of TGFβ might be reflected as increased circulating levels of the cytokine, which might also contribute to the risk of injury (34). It has recently been demonstrated that disruption of the signal transduction pathway for TGFβ (Fig. 2) in transgenic mice deficient in the Smad 3 component of that pathway results in resistance to the development of radiation-induced fibrosis (35). In addition, gene therapy with an adenoviral vector in rats, which results in increased expression of the type 2 TGFβ receptor, reduces tissue levels of TGFβ and protects against radiation-induced injury in the lung (36). Although not yet tested in humans, an anti-TGFβ therapy seems promising as an approach to the prevention of radiation-induced injury.

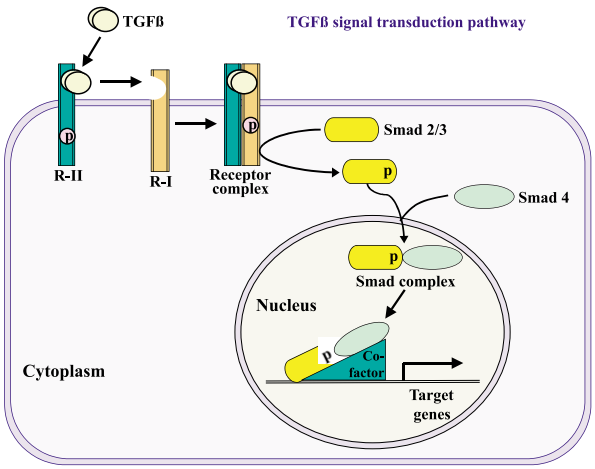

Fig. 2. Simplified version of the transforming growth factor β (TGFβ) signaling pathway. In brief, activated TGFβ binds to the TGFβ type 2 receptor (R-II), which recruits the type 1 receptor (R-I) to form a heterodimeric complex (Receptor complex). This receptor complex is phosphorylated and interacts with the signaling proteins Smad 2 or Smad 3 (Receptor Smad or R Smad). Smad 2 or 3 is phosphorylated, binds with Smad 4, and this complex is translocated into the nucleus, leading to activation of target genes.

As noted above, recent evidence suggests that progressive oxidative stress and hypoxia might be, in part, the driving force behind chronic radiation injury. These data would suggest that long-term therapy aimed at reducing chronic free radical overexpression might be beneficial. One promising approach to this problem involves the superoxide dismutase (SOD) pathway, a naturally occurring mechanism for protection against oxidative damage (37, 38). All three forms of SOD (mitochondrial, cytoplasmic, and extracellular) have been shown to protect against radiation injury (39–41). In humans, the cytoplasmic form has been successfully used to produce regression of established skin fibrosis after radiation (39). Constitutive overexpression of extracellular SOD has been shown to be particularly effective in a mouse model of lung injury, supporting the hypothesis that long-term treatment might be a particularly effective means of preventing injury (41). Compounds that mimic the effect of superoxide dismutase are currently under development, some of which have been shown to have radioprotective properties (9).

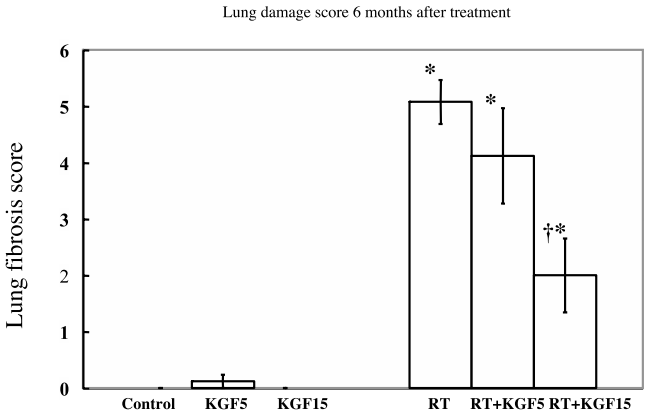

Fig. 3. Lung damage score 6 months after exposure to 40 Gy in 5 fractions to the right hemithorax of Fisher 344 rats. The extent of radiation-induced injury is graded on a previously reported 8-point scale (0 = normal to 8 = severe structural damage). The groups are: untreated control; radiation therapy (RT) alone; keratinocyte growth factor (KGF) alone in doses of either 5 mg/kg (KGF5) or 15 mg/kg (KGF15) IV, given after the last fraction of RT; RT + KGF 5 mg/kg (RT + KGF5); and RT + KGF 15 mg/kg (KGF15). Each group contained 5 animals. Data are presented as mean ± SEM. $^{*}p < 0.05$ compared with control. $^{\dagger}p < 0.05$ compared with RT alone.

NEW DIRECTIONS

Late radiation damage is often characterized histologically by a loss of parenchymal cells and an overproduction of collagen (42). Classic target theory of late radiation injury states that it is the depletion of these parenchymal cells that leads to late injury, and the latent period preceding the development of late effects is due to the long cell cycle time of many of these target cells. This classic concept has been challenged by the finding that radiation triggers a sequence of genetic and molecular events ultimately leading to functional injury (4). Until recently, however, it had not been possible to stimulate repopulation of target cells to determine whether this could reduce acute or late effects. Dorr *et al.* (43) have demonstrated that restoration of the mucosal epithelium with keratinocyte growth factor protects against acute oral mucositis after radiation in animals. Chen *et al.* (44) have found that this same growth factor, which promotes proliferation and differentiation of type II pneumocytes, protects against late radiation-induced lung injury (Fig. 3). Although the mechanism underlying this finding is unclear, it suggests an important role for epithelial–stromal interactions, most likely through alteration of cytokine and/or gene expression, in the genesis of late injury. The molecular biology of these epithelial–stromal interactions warrants further study, because they might provide important new opportunities for designing targeted therapies to prevent normal tissue injury.

In addition to epithelial–stromal interactions, several other pathways are emerging as potential targets for prevention of radiation injury. A detailed discussion of every possible molecular target is beyond the scope of this article, and the reader is referred to *Seminars in Radiation Oncology* 13: 175–380 (2003) for a series of excellent reviews of the topic. A partial list is included in Table 1. Nonetheless, several categories of intercellular signaling molecules implicated in the injury process are worth mentioning. For example, chemokines, which play important roles in inflammation, immunity, and angiogenesis, have been implicated in the development of both idiopathic and bleomycin-induced pulmonary fibrosis (45, 46). In these conditions, pulmonary fibrosis might have resulted from an imbalance between proangiogenic and antiangiogenic stimuli, and restoring this balance might reduce deposition of extracellular matrix (47). Increased expression of chemokines involved in macrophage and lymphocyte recruitment and activation also has been demonstrated in radiation-induced pulmonary fibrosis (48). Whether targeting these molecules can prevent the development of fibrosis after radiation remains to be demonstrated.

Recent evidence has highlighted the importance of microvascular endothelial damage as a major contributor to normal tissue injury after radiation. The endothelium has been shown to be an important target for radiation in the lung, brain, and gut (7, 49). Apoptosis of the microvascular endothelial cell seems to be the earliest lesion in the gut after radiation and leads to stem cell dysfunction (7). Endothelial cell survival factors, particularly basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), have been shown to protect against radiation-induced small bowel injury in animals (7, 50). Although the administration of proangiogenic agents, such as bFGF and VEGF, to cancer patients might prove problematic, other approaches to endothelial cell protection, such as targeting apoptosis through the sphingomyelinase pathway, might prove feasible (7).

Finally, it is evident that radiation injury involves multiple mediators of cellular communication. Agents that target individual mediators specifically, such as antibodies and

Table 1. Partial list of potential molecular targets for the prevention of radiation-induced normal tissue injury

Molecular target	Therapeutic approaches	Status of research
Oxidative stress	Free radical scavengers, SOD isoforms and mimetics, SOD gene therapy	Preclinical studies of SOD mimetics, Phase I studies in development for gene therapy, scavengers in Phase III trials
Cytokines	Anticytokine antibodies, small molecules, gene therapy, nonspecific drugs	Preclinical studies, nonspecific cytokine inhibitors in clinical trials; hematopoetic growth factors and steroids in routine clinical use
Stromal–epithelial interactions (tissue remodeling)	Enhance epithelial cell survival, restoration of damaged epithelium, protease inhibitors	Preclinical studies, clinical trials of the epithelial growth promoter keratinocyte growth factor underway
Chemokines	Antibodies, small molecules, pharmacologic restoration, conditional expression	Preclinical studies
Endothelial cell	Antiapoptotic agents, nonspecific drugs	Preclinical studies, clinical trial of statins underway in Europe
Renin–angiotensin system	ACE inhibitors, AII blockers	Clinical trial underway

Abbreviations: SOD = superoxide dismutase; ACE = angiotensin converting enzyme; AII = angiotensin II receptor.

small molecules, are under development. In the meantime, commercially available compounds, which might have broad activity against multiple cytokines involved in the injury process, are undergoing clinical trials. Examples of such agents include thalidomide and statins; thalidomide inhibits the effects of bFGF, VEGF, TGFβ, and the proinflammatory cytokine tumor necrosis factor α, and statins (3-hydroxy-3-methylglutaryl coenzyme A inhibitors) seem to prevent endothelial cell dysfunction, possibly through maintaining an anticoagulated cell surface (51) and decreasing oxidative stress (52). The results of these trials are anxiously awaited, because new treatments for the prevention and amelioration of radiation-induced normal tissue injury are sorely needed.

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