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.

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-
under investigation (Fig. 1). In many ways, irradiated tissue is known to generate ROS, promote inflammation and vascular permeability, activate the profibrotic cytokine transcriptional machinery (Fig. 1). These changes in the bioavailability of TGF-β 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 TGF-β (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 TGF-β receptor, reduces tissue levels of TGF-β and protects against radiation-induced injury in the lung (36). Although not yet tested in humans, an anti-TGF-β therapy seems promising as an approach to the prevention of radiation-induced injury.

**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 TGF-β 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 TGF-β, which promotes the deposition of fibrous tissue and inhibits epithelial repair (30, 33). These changes in the bioavailability of TGF-β 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 TGF-β (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 TGF-β receptor, reduces tissue levels of TGF-β and protects against radiation-induced injury in the lung (36). Although not yet tested in humans, an anti-TGF-β therapy seems promising as an approach to the prevention of radiation-induced injury.

![Diagram of the TGF-β signal transduction pathway](image)

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This diagram illustrates the signal transduction pathway for TGF-β. In brief, activated TGF-β binds to the TGF-β 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 signal-transducing 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).

**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

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>Therapeutic approaches</th>
<th>Status of research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>Free radical scavengers, SOD isoforms and mimetics, SOD gene therapy</td>
<td>Preclinical studies of SOD mimetics, Phase I studies in development for gene therapy, scavengers in Phase III trials</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Anticytokine antibodies, small molecules, gene therapy, nonspecific drugs</td>
<td>Preclinical studies, nonspecific cytokine inhibitors in clinical trials; hematopoietic growth factors and steroids in routine clinical use</td>
</tr>
<tr>
<td>Stromal–epithelial interactions (tissue remodeling)</td>
<td>Enhance epithelial cell survival, restoration of damaged epithelium, protease inhibitors</td>
<td>Preclinical studies, clinical trials of the epithelial growth promoter keratinocyte growth factor underway</td>
</tr>
<tr>
<td>Chemokines</td>
<td>Antibodies, small molecules, pharmacologic restoration, conditional expression</td>
<td>Preclinical studies</td>
</tr>
<tr>
<td>Endothelial cell</td>
<td>Antiapoptotic agents, nonspecific drugs</td>
<td>Preclinical studies, clinical trial of statins underway in Europe</td>
</tr>
<tr>
<td>Renin–angiotensin system</td>
<td>ACE inhibitors, AII blockers</td>
<td>Clinical trial underway</td>
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</tbody>
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Abbreviations: SOD = superoxide dismutase; ACE = angiotensin converting enzyme; AII = angiotensin II receptor.