Target-Based Interventions to Treat Radiation-Induced Lung Injury

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KEY POINTS

- Delivery of the therapeutically optimum dose of radiation to achieve local tumor control and improved survival is limited by the risk of unacceptable normal tissue toxicity.
- Improved understanding of the molecular mechanisms underlying radiation-induced normal tissue injury has identified a number of molecular pathways, pro-fibrogenic growth factors, inflammatory cytokines, and chronic oxidative stress that can be targeted to ameliorate normal tissue injury.
- Approaches to prevent, mitigate, and/or treat radiation-induced normal tissue injury have been investigated in pre-clinical and clinical studies with varying degrees of success.
- Better understanding of normal tissue radiobiology will lead to improved therapeutic targeting to prevent, mitigate, and/or treat radiation-induced normal tissue toxicity.
- Therapeutic modalities to prevent, mitigate, and/or treat radiation-induced normal tissue toxicity will allow a higher clinically achievable therapeutic dose, improve local tumor control, diminish the negative consequences of radiation toxicity on quality of life, and potentially improve overall patient survival.

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Abstract

The ability to achieve local tumor control and improved overall survival with radiation therapy is limited by the risk of unacceptable normal tissue toxicity. A number of therapeutic interventions targeting the molecular pathways responsible for the development of acute and long-term injury have been investigated in pre-clinical and clinical studies. These interventions have primar-

ily targeted apoptosis, growth factors, and pro-inflammatory and pro-fibrogenic pathways in an attempt to prevent, mitigate, or treat radiation-induced injury. As the mechanisms underlying radiation-induced normal tissue injury are better elucidated, the identification of new potential targets and improved therapeutic interventions will allow patient-stratified dose escalation and improve long-term response rates. The following chapter outlines current target-based interventions being investigated and discusses the recent discovery of novel pathways that may be targeted in the future.

12.1 Introduction

The number of cancer survivors in the US alone now exceeds 10 million people. As the number of cancer survivors increases, the percentage of survivors with chronic health conditions related to treatment toxicity has grown. For the majority of patients diagnosed with cancer, radiation therapy with curative or palliative intent will be an important component of treatment. The likelihood for developing radiation-induced normal tissue toxicity has broad implications not only for the cancer survivor population, but also for patients in which more aggressive cytotoxic treatment strategies might be beneficial both for achieving tumor control and improvement in survival. Thus, the probability for normal tissue complications during or after radiotherapy limits the maximum effective dose that can be delivered to the tumor (Stone et al. 2003). The maximum tolerated dose is unknown for most tissues, and the ability to predict individual patient risk for the development of delayed injury is challenging. Formal dose escalation studies in radiation oncology have rarely been done. Thus, the clinically accepted tolerated doses have been mostly empirically derived through clinical observation and experience (Emami et al. 1991; Milano et al. 2007), and these doses are well below those required to achieve local control for most solid tumors. While these tolerance doses may be reasonable estimates for large populations of patients, the radiosensitivity of an individual cannot be determined accurately at this time.

The clinical significance of this problem can best be illustrated using the example of non-small cell lung cancer (NSCLC). Non-small cell carcinoma of the lung (NSCLC) is one of the most common cancers in the United States, with more than 172,000 cases diagnosed in 2005 (Jemal et al. 2005). It remains the most common cause of cancer deaths in this country. About one-third of these patients will present with non-metastatic, but locally advanced or medically inoperable disease. For these patients, radiation therapy has been the mainstay of treatment.

Despite the high frequency of distant metastases in patients with unresectable NSCLC, local failure remains a significant clinical problem. The Radiation Therapy Oncology Group (RTOG) trials have reported radiographically assessed local failure rates of 35–58% in this patient population (Perez et al. 1986). Arriagada et al. (1991) reported on a series of 353 patients more rigorously assessed for local control following either radiotherapy alone (65 Gy in 2.5-Gy fractions) or the same radiotherapy plus chemotherapy (vindesine, cyclophosphamide, cisplatin, and lomustine) for unresectable NSCLC. In this randomized trial, patients underwent routine bronchoscopy and biopsy 3 months after radiotherapy and at 6-month intervals thereafter. The actuarial local control rate at 1 year was only 15–17% with radiotherapy + chemotherapy; chemotherapy did not improve local control.

Several investigators have noted the importance of local control in the treatment of NSCLC. In patients with unresectable NSCLC, Perez et al. (1986) demonstrated that intrathoracic tumor control at 6 months following radiotherapy predicted improved survival. These authors reported a 20% 3-year survival in complete responders compared to 4% in partial responders. Saunders et al. (1984) noted a similar finding, with 65% of complete responders alive at 2 years, compared to none of the partial responders. The uncertainties in defining local control in unresectable lung cancer make it difficult to be certain that improvements in local control are achievable with higher radiation doses and that this reduction in local relapse will translate into an increase in survival. Nevertheless, the available data suggest that the total tumor dose appears to significantly influence both local control and survival in NSCLC. The RTOG reported local failure rates of 53%, 49%, and 35% for patients irradiated to total doses of 40 Gy, 50 Gy, and 60 Gy, respectively (Perez et al. 1986). Median survivals in these three groups were 7 months, 9 months, and 10 months, respectively. Other investigators have confirmed the findings of Perez. For example, when controlling for tumor size, Dosoretz et al. (1993) noted a better disease-free survival for doses above 65 Gy versus doses of 60–65 Gy. As a result of findings, investigators at several institutions have instituted pilot, phase I, and phase II radiation dose escalation studies in patients with NSCLC (Cox et al. 1990; Anscher et al. 2001; Roseman et al. 2002; Zhao et al. 2007; Socinski et al. 2008). Much higher doses have been achieved using the

modern radiotherapy techniques of three-dimensional conformal (3DCRT), intensity-modulated radiotherapy (IMRT) and image-guided adaptive radiation therapy (IGRT) (Robertson et al. 1997; Keall et al. 2006; Schild et al. 2006). Despite the highly sophisticated approaches to radiation dose delivery utilized in these studies, normal tissue injury remains a significant problem.

Certain factors affecting normal tissue tolerability include the anatomic location of the tumor, the cellular kinetics and organization of surrounding tissue, preexisting pathologic conditions, genetic variability, and physical factors (fractionation scheme, total dose, and volume irradiated). The effect of normal tissue complications on the physical and emotional quality of life for cancer survivors depends on the tissue affected and severity of injury. For a minority of these patients, the long-term effect of treatment is worse than the disease itself. As the number of long-term cancer survivors increases, late complications of cancer therapy are becoming an increasingly important concern to both physicians and patients. For the radiation oncologist, a better understanding of the molecular events underlying normal tissue injury will permit a more rational approach to its prevention and treatment (Anscher et al. 1994). Currently, the physician must try to prevent complications primarily through restricting the dose and volume to be irradiated (Vujaskovic et al. 2000). The relationship between dose, volume, complications, and tumor control is complex and not precisely defined for most cancers and normal tissues (Emami et al. 1991; Vujaskovic et al. 2000; Milano et al. 2007). Only recently have investigators attempted to better delineate these relationships by taking advantage of innovations in radiation dose delivery and imaging technology.

The relationship between the probability of local tumor control and probability for normal tissue toxicity is defined by the therapeutic ratio (Fig. 12.1). Both have sigmoid dose response curves, and a number of therapeutic interventions have been investigated to either improve tumor sensitivity to radiation or reduce the deleterious effects of radiation on normal tissue without negatively affecting tumor response (Brizel 2007). Treatment options for radiation-induced injury are defined as (1) prophylactic agents, typically given prior to irradiation, (2) mitigators, agents given after irradiation, but prior to symptomatic injury, or (3) treatment given at the time of symptomatic injury to reverse tissue damage (Moulder 2003).

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. It has been known for decades that the biologic response to ionizing radiation begins with the generation of reactive oxygen species (ROS) (Riley 1994). More recently, researchers have described how these immediate biochemical events rapidly trigger a series of genetic and molecular phenomena leading to clinically and histologically recognizable injury (Brach et al. 1991; Barcellos-Hoff 1993; Hong et al. 1995; Johnston et al. 1995; Rubin et al. 1995; Hauer-Jensen et al. 1999; Vujaskovic et al. 2001; Hallahan et al. 2002; Hong et al. 2003). This response to radiation is dynamic and involves a number of mediators of inflammation and fibrosis produced by macrophages, epithelial cells, and fibroblasts. These events appear to be sustained for months to years beyond the completion of therapy; however, the mechanisms responsible for maintaining the injured phenotype, until recently, have remained unknown (Vujaskovic et al. 2001) .

As in tumor biology, the improvement in knowledge of ongoing molecular processes in radiation-induced injury has provided new mechanisms and insights for modulating the cellular and tissue response using target-based strategies to ameliorate normal tissue injury. This chapter will review potential molecular and physiological targets and respective therapeutic interventions, focusing on protection of normal tissues as a model for translational studies for target-based therapies.

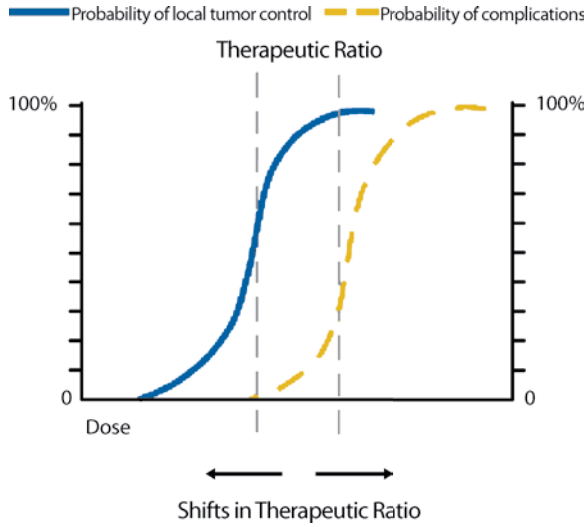

Fig. 12.1. Therapeutic interventions seek to improve the therapeutic ratio by increasing normal tissue tolerance to radiation or through radiosensitization of the tumor. Reproduced from Brizel (2005)

12.2 Molecular and Physiological Basis of Normal Tissue Injury

It was originally assumed normal tissue injury was an unavoidable, untreatable, and irreversible consequence of radiation therapy. The classical radiobiology concept supported the target cell hypothesis, which suggested the effects of radiation were mostly the result of clonogenic cell death (Michalowski 1984). The acute versus late tissue effects therefore corresponded to the rate of cell turnover in the irradiated tissue (Michalowski 1984). However, since the early to mid-1990s, the target cell hypothesis has come under increasing scrutiny. Although cell kinetics play a role in radiation-induced injury, it has now been acknowledged that radiation-induced normal tissue injury is a consequence of dynamic interactions among various cells of the tissue, inflammatory mediators, and the vascular endothelium beginning at the time of irradiation and continuing throughout the time to disease progression (Rubin et al. 1995; Vujaskovic et al. 2001; Fleckenstein et al. 2007b). This is particularly relevant for late-responding tissue (lung, liver, and spinal cord) in which there is a latent period lasting months to years before which symptoms may appear.

The acute phase of radiation injury develops during the course of radiation therapy or immediately after and is primarily characterized by injury to tissues with rapidly proliferating stem cell compartments such as the salivary glands (xerostomia), gastrointestinal mucosa (mucositis, diarrhea), or skin (erythema). Likewise, late injury develops in tissues with slow proliferation indexes (lung, liver, and CNS). The new molecular-based paradigm has renewed interest to develop therapeutic interventions for radiation-induced normal tissue toxicity to target the series of ongoing events leading to the development of symptomatic injury.

The biological effects of ionizing radiation begin with the transient increase in reactive oxygen species (ROS), such as superoxide ($O2^{-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$) at the time of irradiation (Riley 1994). The direct effect is injury to cellular components, chromosomal damage, and cell-mediated death (Rubin et al. 1995). Within days post-radiation, there are noticeable effects on vascular endothelial function, including increased leukocyte-endothelial interac-

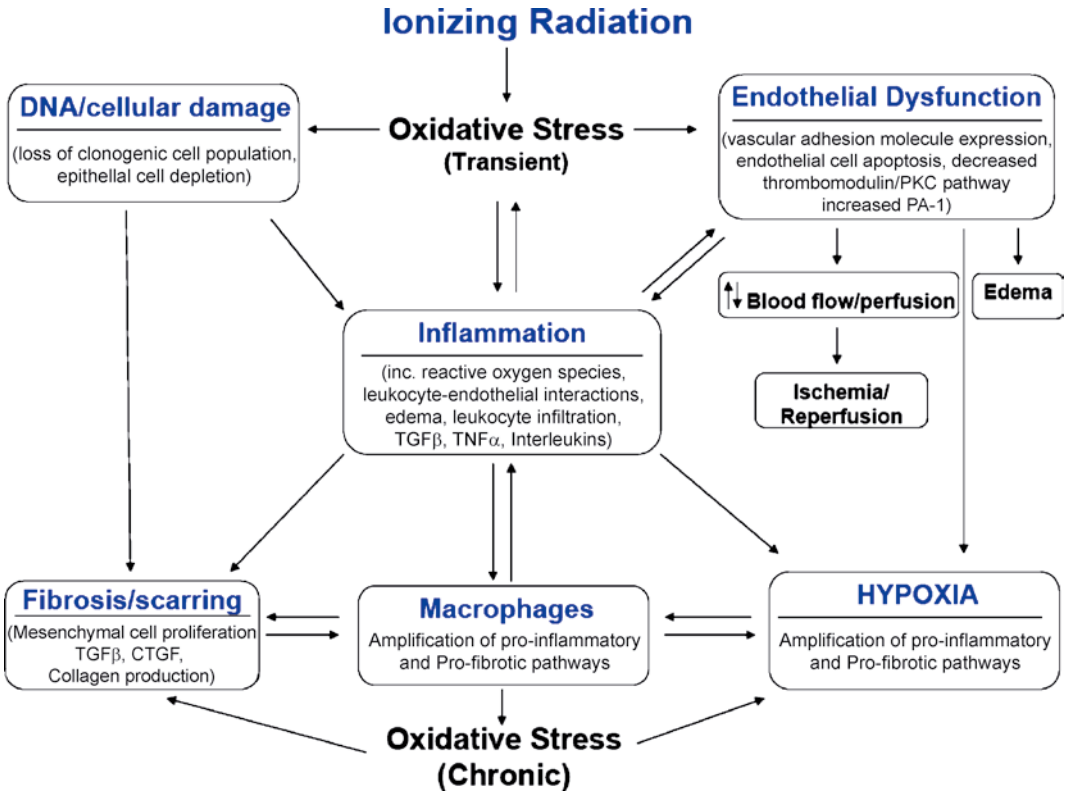

Fig. 12.2. Simplified diagram of the molecular mechanisms underlying radiation-induced injury

tions, detachment of endothelial cells from the basal lamina, endothelial cell apoptosis, and loss of microvessel density, resulting in reduced blood flow and tissue hypoxia (Dewhirst et al. 1987; Wang et al. 2007a). The immediate cellular injury and vascular dysfunction are followed by an acute and progressive inflammatory cell infiltration and activation of an ongoing cytokine cascade, leading to chronic oxidative stress. These cellular events are ongoing throughout the "latent" period prior to the development of symptomatic injury. The end result is an environment characterized by endothelial dysfunction, extravasation of plasma proteins and edema, inflammatory cell infiltration and activation, lipid peroxidation, fibrin accumulation in the extracellular matrix, and functional tissue damage (Fig. 12.2) (Stone et al. 2003; Brush et al. 2007; Delanian et al. 2007; Rodemann et al. 2007b; Zhao et al. 2007).

A number of cytokines, growth factors, and oxidant-generating enzymes have been implicated in the aforementioned processes and have been used as potential targets in an attempt to ameliorate and/or treat radiation-induced normal tissue injury. A growing body of evidence points toward a complex web of protein interactions as being important in the pathogenesis of abnormal fibrogenesis (see Table 12.1). For example, Huang et al. (2002) have found that IL-7, a cytokine that enhances T cell function and IFN-γ production, inhibits both TGFß production and signaling, and protects against the development of bleomycin-induced pulmonary fibrosis. Fedorocko et al. (2002) showed that radiation exposure could increase cytokine production both directly (IL-6, TNF-α) and indirectly (GM-CSF), either by locally acting paracrine or endocrine effects or as a result of systemic effects of early proinflammatory mediators such as IL-1 or TNF-α. There is no doubt that protein production is a dynamic process, which will change as a result of cancer treatment. Hong et al. (2003) have documented temporal and spatial changes in the expression of proinflammatory cytokines (TNF-α, IL-1α, and IL-1ß) following thoracic irradiation in mice.

Table 12.1 lists proteins that might be potential targets for intervention, since they are components in all of the major pathways thought to be involved in the response of cells to radiation (Schmidt-Ullrich et al. 2000; Tsoutsou et al. 2006).

Table 12.1. Summary of the function of candidate proteins

Protein	Function
IL-1ß	Inflammation, growth factor expression
IL-5	Proinflammatory
IL-6	Proinflammatory, decrease apoptosis of activated lung fibroblasts
IL-7	Proinflammatory
IL-8	Angiogenesis, leukocyte chemotaxis, collagen synthesis
IL-10	Anti-inflammatory (decrease TNFα production, decrease upregulation of endothelial cell adhesion molecules)
IL-13	Proinflammatory
MCP-1	Inflammation, chemoattraction of monocytes
MIP-1alpha	Antiproliferative
PDGF BB	Angiogenesis, recruit smooth muscle cells
VEGF	Angiogenesis and increased vascular permeability
EGF	Epithelial cell motility, mitogenicity, and differentiation
EGFR	Receptor for EGF, initial component of EGF signaling pathway
NFkappaB	Pleotropic gene transcription responses
HIF-1	Transcription factor for genes regulating angiogenesis

Table 12.1. (*continued*) Summary of the function of candidate proteins

Protein	Function
TGF-alpha	Cell motility and proliferation
FGF 2	Angiogenesis and fibroblast proliferation
MMP-1	Degradation of collagen and extracellular matrix proteins
MMP-2	Matrix remodeling, growth factor release
MMP-3	Matrix remodeling, growth factor release
MMP-13	Matrix remodeling, growth factor release
SMAD 2/3	Signal transduction in the TGFß pathway
IGF-1R	Binding of IGF-1 (reepithialization and granulation tissue formation)
TNF-alpha	Growth factor expression, inflammation, matrix production, and remodeling
TGFß	Profibrotic, immunosuppression, angiogenesis, metastasis
Beta-catenin	Epithelial-mesenchymal transition
Nitric-oxide synthases	Inflammation
Superoxide dismutases	Endogenous anti-inflammatory regulator

12.3 Target-Based Therapeutic Strategies

12.3.1 Transforming Growth Factor Beta (TGFβ)

Over the past 20 years, the role of TGFβ in post-radiation injury has been extensively studied in experimental models of radiation-induced cellular and tissue injury. Since Martin et al. (2000) described TGFβ signaling to be the *master switch* in radiation-induced fibroproliferative disease, investigators have sought to mitigate the severity of injury through TGFβ targeting. TGFβ, a pleuripotent cytokine, is a critical mediator of cell growth and proliferation, extracellular matrix remodeling, inhibition of matrix degradation, chronic inflammatory disease, and angiogenesis (Roberts 1999; Flanders 2004). Latent TGFβ is sequestered in the extracellular environment until it is activated by proteases, free radicals, or radiation (Barcellos-Hoff et al. 1996). The latency-associated peptide (LAP) bound to TGFβ acts as a molecular chaperone and sensor of oxidative stress (Baecellos-Hoff 1993; 1996; Barcellos-Hoff et al. 1994, 1996; Vodovotz et al. 1999; Jobling et al. 2006). Recently, Jobling et al. (2006) used free radical scavengers to determine that hydroxyl radical bioavailability, which can be produced by radiolytic hydrolysis, is the primary oxidizing agent responsible for activation of latent TGFβ. It is therefore plausible to assume that oxidation of LAP explains the rapid increase in active TGFβ observed within hours post radiation (Fleckenstein et al. 2007a). The biologically active form of TGFβ readily binds the ubiquitously expressed TGFβ type I and II receptors (Roberts 1999; Flanders 2004; Andrawewa et al. 2007a, 2007b). Stabilization of the type II/type I receptor complex by their cytoplasmic domains leads to downstsream phosphorylation of Smad 2/3 proteins, which then form an active heterooligomeric complex with Smad 4 that can bind DNA and initiate transcription (Fig. 12.3) (Roberts 1999).

In a recent paper by Fleckenstein et al. (2007b), 28-Gy single-dose irradiation to the right hemithorax resulted in increased TGFβ production within 24 h post-radiation followed by a bi-phasic decrease in perfusion, development of tissue hypoxia, and infiltration and accumulation of macrophages with a concomitant increase in oxidative stress.

Other studies aimed at identifying TGFβ as a key mediator in the pathological response to radiation have used antagonists to TGFβ or components of its signal transduction pathway (Rabbini et al. 2003; Nishioka

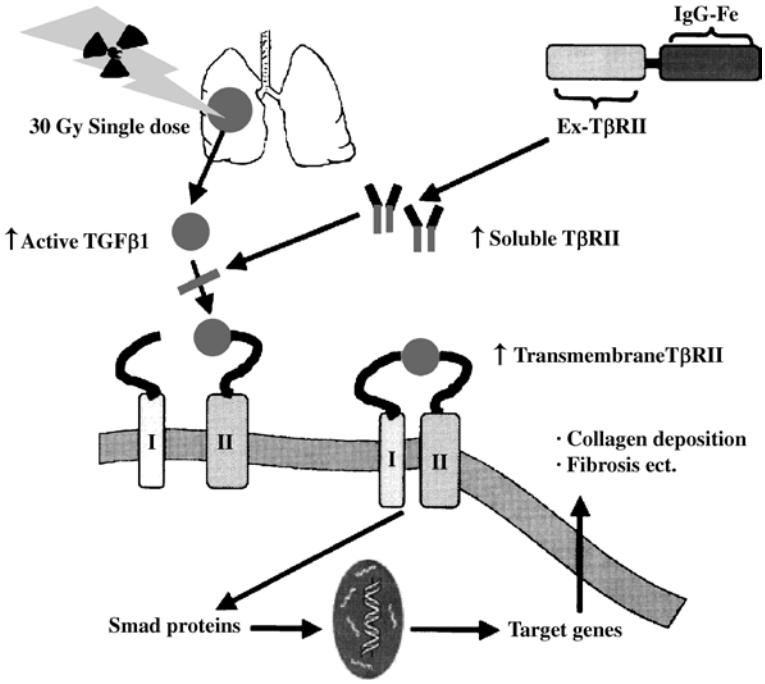

Fig. 12.3. TGFβ signaling pathway and therapeutic targeting with soluble TGFβ receptor (TβRII). Reproduced from Rabbani et al. (2003)

et al. 2004; Anscher et al. 2006, 2008). Anscher and Vujaskovic (2008) found a small molecule kinase inhibitor targeting the TGFβ pathway preserved the structural integrity of the lung and prevented organ dysfunction using both biological and functional parameters to assess the severity of lung injury after 28-Gy single-dose right hemithoracic irradiation. Their 2007 study was consistent with studies conducted between 1995 and 2006 (Ehrhart et al. 1997; Nishioka et al. 2004; Anscher et al. 2006) in which several authors found blockade of the TGFβ signaling pathway using an anti-TGFβ antibody or adenoviral vector expressing a soluble TGFβ receptor to neutralize the protein in vivo significantly protected lung tissue from radiation injury. In Anscher and Vujaskovic's studies (2006), histological and morphologic comparison among animals at the end of the follow-up period (6 months) showed a decrease in macrophage infiltration and inflammation, reduced alveolar wall thickness and collage deposition, and an improvement in overall lung function (Rabbini et al. 2003). These results are supported by Nishioka and colleagues (2004), who reported similar findings using an adenoviral vector expressing soluble TGFβ receptor in their experimental model of hemithoracic lung injury using 30-Gy single-dose irradiation.

12.3.2 Keratinocyte Growth Factor

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family. KGF is produced by mesenchymal cells (i.e., fibroblasts, γδT-cells) and is specific for epithelial cells expressing a splice variant of FGFR2. KGF stimulates proliferation and differentiation to facilitate re-epithelialization of injured tissue. A number of studies have sought to mitigate radiation-induced injury through modulation of cellular proliferation, particular for acute responding tissues, such as the oral mucosa. The best data for the utility of KGF in mitigation or treatment of radiation-induced injury come from animal models of oral mucositis, a common complication from treatment of the head and neck cancer. Dorr and colleagues (2001, 2002, 2005c) in Dresden have provided much of the evidence regarding the protective effect of rHuKGF in pre-clinical settings using both single-dose and fractionated radiation. In a 2005 study, Dorr found a dose-response effect of rHuKGF (palifermin) in a mouse model of oral mucositis following fractionated irradiation. The highest ED50 values for mucosal tolerance were achieved with 15 mg/kg or 22.5 mg/kg; however, the authors found doses as low as 1 mg/kg offered significant protection against mouse tongue ul-

ceration (Dorr et al. 2005b). rHuKGF given during the course of radiotherapy appears to be more effective in models of oral mucositis rather than when given prior to the start of radiation treatment. Studies by Dorr suggest doses above 30 mg/kg offer no improvement over those seen at 15 and 22.5 mg/kg (Dorr et al. 2005b). In those studies, the optimum therapeutic strategy involved three applications of rHuKGF during the course of radiation therapy; however, greater than three did not offer any significant improvement (Dorr et al. 2005c). Thus, it appears there is a cumulative dose threshold for which no greater protection is achieved with higher doses or further treatment. The radioprotective effect was preserved when chemotherapy (5-FU and cisplatin) was combined with radiation (Dorr et al. 2005a).

KGF has been shown to be a direct stimulator of type II pneumocytes both in vitro and in vivo (Ulich et al. 1994; Terry et al. 2004). Chen et al. (2004) found high doses of recombinant human KGF (15 mg/kg) given i.v. 15 min prior to the last fraction of radiation offered significant protection against radiation-induced pulmonary injury. The protective effect was assessed using functional injury (breathing frequency) and histological, morphological, and immunohistochemical staining for architectural/structural distortion, collagen deposition, and activation of the TGFβ/Smad signaling pathway. The protective effect of rHuKGF on the lung is described to be a result of type II pneumocyte differentiation to type I pneumocytes, which normally comprise 95% of the alveolar surface area, concurrent with apoptosis of hyperplastic alveolar type II cells (Fehrenbach et al. 1999, 2000, 2002). Terry et al. (2004) described an actively proliferating pulmonary environment after stimulation with rHuKGF. Terry found whole thorax irradiation to mice at the time of increased alveolar epithelial cellularity (after KGF delivery) resulted in a right shift in the dose response curve for radiation-induced pneumonitis. Jaal and Dorr recently completed a new study to investigate the effect of rHuKGF on mouse urinary bladder. A single dose of 15mg/kg given subcutaneously prior to irradiation reduced both the early and late effects of bladder toxicity. The same effect was not observed when given after irradiation (Jaal et al. 2007); however, longer administration of the drug post-irradiation may provide better results.

12.3.3 Angiotensin-Converting Enzyme (ACE)

The success of angiotensin-converting enzyme inhibitors and AngII receptor antagonists for reducing the development of late injury has been shown in a number of published studies during the past 2 decades (Moulder et al. 1993, 1996, 197a, 1997b, 1998a, 1998b, 1998c, 2003, 2007b; Cohen et al. 1997; Molteni et al. 2000; 2007). Indirect evidence for the role of a renin-angiotensin system in radiation-induced delayed injury stems from studies using ACE inhibitors to successfully reduce the severity of delayed radiation nephropathy; however, no evidence of alterations in either the enzyme renin or its substrate, angiotensin II, have been found.

The utility of ACE inhibitors has been investigated primarily in animal models of radiation nephropathy. Radiation nephropathy is characterized by tubulointerstitial fibrosis and glomerulosclerosis, leading to renal failure (Robbins et al. 2006). Within 5 weeks after bilateral or total body irradiation followed by bone marrow transplant, there is an increase in the number of cells staining positive for proliferating cell nuclear antigen, suggesting irradiation induces an increase in renal tubular cell proliferation. It was thought cellular proliferation or chronic oxidative stress may be a target for ACE inhibitors and AII blockers (Moulder et al. 2002). However, non-thiol-containing ACE inhibitors, such as enalapril, were also effective at reducing the severity of radiation-induced renal injury diminishing the enthusiasm for chronic oxidative stress as the underlying target. The thiol-containing captopril was one of the first ACE inhibitors proven to both prevent and treat radiation nephropathy. Moulder and colleagues (1998a) found the actuarial risk of renal failure after bilateral irradiation in a rat model was significantly reduced with continuous treatment of Captopril (62.5 mg/l and 500 mg/l) or AII blocker (L 158,809) when treatment started 24 weeks post exposure during the time of established injury. Moulder and colleagues (1998b) demonstrated the protective effect of Captopril was independent of the pharmacologic dose and found low doses achieved equally effective mitigation of nephropathy assessed by a decrease in azotemia, proteinurea, and histopathologic damage. The same group determined the better efficacy of AII blocker as a prophylactic agent compared to Captopril was suggestive of dual mechanisms underlying the acute and delayed renal responses to radiation. At doses below the human MTD, Captopril increased survival from 49 weeks (irradiated alone) to 74 weeks ($P < 0.0001$). In these studies, Captopril or AII blocker was only effective when given continuously after the development of injury. In subsequent studies, the authors found ACE inhibitors delivered for short time intervals (3-6 weeks) could be effective when delivered between 3 to 10 weeks post-irradiation, before or after which the effectiveness diminished (Cohen et al. 1997; Moulder et al. 1998b), coinciding with the time of renal tubular cell and glomerular proliferation. However, in those

same studies, Moulder and colleagues found that AII blockers inhibited renal tubular cell proliferation, but had no effect on glomerular cell proliferation. Consequentially, in experimental models of radiation-induced pneumonitis and fibrosis, cessation of treatment with ACEI has been followed by a rapid deterioration in lung injury (Robbins et al. 2006).

Based on promising animal work, Cohen et al. (2008) launched a phase III trial of Captopril vs. placebo after hematopoeitic stem cell transplant to mitigate chronic renal failure. The study included both adults and children with various types of leukemia or myelodysplastic syndrome. Patients received total body irradiation to 14 Gy in nine fractions, with the dose to the kidney limited to 9.8 Gy. Captopril was started after engraftment was confirmed, beginning at a dose of 6.25 mg b.i.d. and escalating to a dose of 25 mg t.i.d. (12.5 mg t.i.d. in children). Unfortunately, the study was unable to meet its accrual goals. Despite this problem, however, there was a trend toward better preservation of renal function in the Captopril-treated group, as measured by glomerular filtration rate at 1 year ($P = 0.07$). Thus, this study supports the conclusion that an ACE inhibitor may mitigate chronic renal failure after radiation-based hematopoeitc stem cell transplant. This study awaits confirmation.

12.3.4 Statins

The molecular and cellular events leading to late toxicity after RT begin virtually immediately after the first exposure to ionizing radiation. Endothelial cell damage plays an important role in this process, and recent evidence suggests that the capillary endothelial cell may be the first cellular element to be damaged by RT (Paris et al. 2001). Late vascular effects include, in addition to telangiectasia development, capillary collapse, thickening of the basement membrane, and loss of clonogenic capacity (Pena et al. 2000). Capillaries also may be the most sensitive component of the vascular system (Rodemann et al. 2007a). Vascular damage is important in the phenotype of RT-induced rectal injury, where telangiectatic vessels are often responsible for the bleeding characteristic of this condition (Garg et al. 2006).

The molecular pathways involved in endothelial cell death probably involve both DNA damage-dependent and -independent mechanisms (Fig. 12.4). At higher doses (10–20 Gy), radiation-induced apoptosis, mediated through the generation of ceramide via the sphinomyelin pathway (Li et al. 2003), appears to be the dominant mechanism. Ceramide mediates the activation of three major pathways of endothelial cell apoptosis: the MAPK 8 pathway, the mitochondrial pathway, and the death receptor (TNF) pathway (Rodemann et al. 2007a). The MAPK 8 pathway seems dominant, and this pathway results in apoptosis through the action of effector caspases (Rodemann et al. 2007a). Similar processes are thought to mediate a number of other chronic conditions, including coronary artery disease (Forrester et al. 2007).

The cholesterol-lowering agents 3-hydroxy-methyl-glutaryl Co-A reductase (HMG Co-A reductase) inhibitors (statins) have been demonstrated to reduce the risk

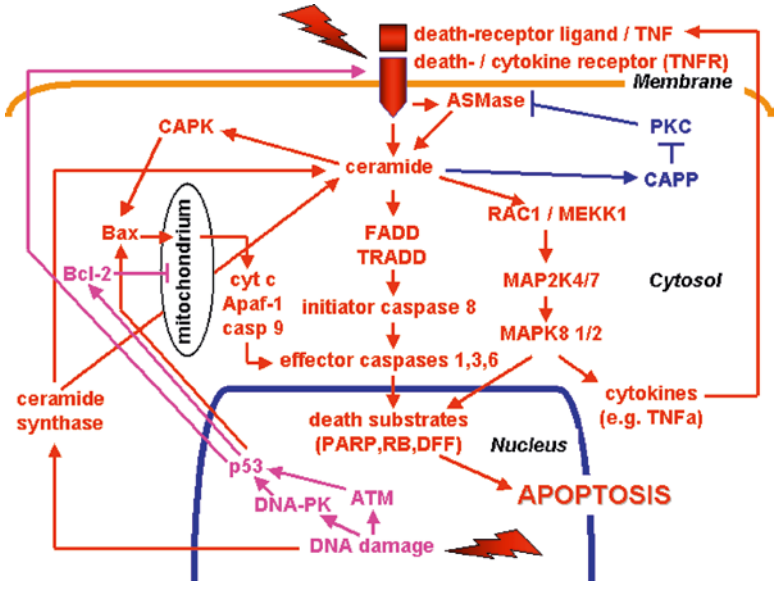

Fig. 12.4. Pathways of endothelial cell apoptosis following exposure to ionizing radiation. Reproduced from Rodemann (2007a)

of myocardial infarction, in part, through their vascular protective effects, which are not dependent on changes in serum cholesterol levels (ROSENSON 2001). In vitro, statins have been shown to inhibit the expression and/or activity of mediators of inflammation, including reactive oxygen species, TNFα, cyclooxygenase-2, matrix metalloproteinases, and thromboxane A2, while increasing the expression of anti-inflammatory effectors, such as nitric oxide synthase (FORRESTER et al. 2007).

Emerging evidence suggests that statins may afford protection against the deleterious effects of ionizing radiation. In vitro, statins have been shown to protect human endothelial cells from ionizing radiation (GAUGLER et al. 2005; BOERMA et al. 2006; NUBEL et al. 2006). Multiple mechanisms appear to be involved, including attenuation of extracellular stress responses (RIKITAKE et al. 2001; MORIKAWA et al. 2002), down-regulation of chemokines and chemokine receptors (WAEHRE et al. 2003), and by exerting anti-inflammatory and antithrombotic effects (UNDAS et al. 2002; PEREZ-GUERRERO et al. 2003; SHI et al. 2003; BOERMA et al. 2006) on these cells. After irradiation, there is an early increase in pro-inflammatory cytokines (IL-6, TNF-α) and transcription factors (NFκB) leading to the development of lymphedema and tissue fibrosis. Statins have been shown to reduce vascular endothelial cell activation and inflammatory cytokine and transcription factor production, specifically IL-6, TNFα, and NFκB (HAYDONT et al. 2007a; PARK et al. 2008a, 2008b).

The biological rationale for statins as an interventional approach to mitigate radiation-induced injury has recently been postulated to result from inhibition of the Rho/Rock pathway, which exerts influence over vascular function and pro-inflammatory and pro-fibrotic cytokines (HAYDONT et al. 2007b). Gene arrays of irradiated tissue have shown divergent expression of genes coding for the Rho/Rock pathway from normal tissue (BOURGIER et al. 2005). Furthermore, BOURGIER and colleagues (2005) treated isolated primary intestinal smooth muscle cells from ileal biopsies taken from patients with late radiation enteritis with Rho inhibitor to determine whether it could alter the cells pro-fibrogenic phenotype. Inhibition of the Rho pathway decreased expression of both connective tissue growth factor and collagen type I. The decrease in fibrogenic activity was in contrast to isolated untreated cells, which showed cytoskeletal rearrangement, alteration in Rho pathway gene expression, and increased connective tissue growth factor (CTGF) and collagen secretion. Thus, the ability of statins to reduce inflammation and fibrotic activity (including downregulation of CTGF) when given post-radiation suggests potential mediation through the Rho/ROCK pathway. In a subsequent study by HAYDONT et al. (2005), Pravastatin, a hydrophilic statin, reduced CTGF, TGFβ, and collagen production from intestinal smooth muscle cells isolated from patients with radiation enteritis and improved radiation enteropathy in an animal model (HAYDONT et al. 2007a).

Two independent investigators found statins had a limited effect on the early, acute effects of radiation on normal intestine; however, they significantly ameliorated delayed injury, resulting in less collagen deposition and reduced mucosal injury (HAYDONT et al. 2007c; WANG et al. 2007b). HAYDONT et al. further evaluated the effect of Pravastatin on three tumor cell lines in vivo and demonstrated no protective effect on tumor response to radiation. Thus, statins may have the potential to protect against RT-induced late effects, and, in fact, atorvastatin is currently being evaluated as a treatment to prevent progression of carotid artery intima-media damage after RT to the head and neck in the Netherlands (F. Stewart, personal communication).

12.3.5 Pentoxifylline and *α*-Tocopherol

A number of successful clinical trials carried out over the last decade have demonstrated treatment with Pentoxifylline (PTX) and alpha-tocopherol (vitamin E) during the course of radiation therapy and up to 2 years thereafter reverses superficial fibrosis and mitigates lung injury. DELANIAN and colleagues (1999) enrolled 52 patients with symptomatic radiation-induced superficial fibrosis between 1995 and 1997. Patients were treated with a combination of 400 mg PTX and 500 IU vitamin E twice per day for 1 year after the development of fibrosis. Significant regression (mean RIF surface area) and functional improvement (SOMA) were observed 3 months to 1 year after the start of treatment with PTX-vitamin E. No unacceptable toxicity with PTX and vitamin E was observed in any of the enrolled patients. The precise mechanism of action of PTX and vitamin E is unknown; however, multiple pre-clinical and clinical studies have shown combined treatment is more effective than either given alone, suggesting a synergistic mechanism of action (LEFAIX et al. 1999). In a follow-up study published in 2005 in the *Journal of Clinical Oncology*, DELANIAN and colleagues (2005) compared short-term (6 to 12 months) versus long-term (24 to 48 months) treatment of symptomatic radiation-induced fibrosis with PTX-vitamin E. Patients receiving PTX-vitamin E had a 68% reduction in fibrosis at 24 months. However, the authors found recurrence of

radiation-induced fibrosis when PTX-vitamin E treatment was discontinued (6 to 12 month treatment arm). The most significant and long-term regression of fibrosis occurred in patients treated for 3 or more years. The same year, Haddad et al. (2005) published the results of a phase II clinical trial with 34 patients treated for 3 months with 800 mg PTX and 1,000 U vitamin E daily for superficial radiation-induced fibrosis. Patients were followed for fibrotic surface area regression and grade of fibrosis (SOMA scale). Out of the 29 patients who completed the study, the mean surface area regression at 3 months had decreased by 43% (±19%; $P < 0.001$). Eighteen patients who continued on PTX-vitamin E for 6 months had a 72% (±15%) reduction in surface area. Misirlioglu et al. (2007) evaluated the radioprotective benefit of PTX-vitamin E in lung cancer patients receiving thoracic irradiation. Forty-four patients received 400 mg PTX three times per day and vitamin E 300 mg twice per day during the course of radiation therapy and thereafter for 3 months. Patients treated with PTX-vitamin E had significantly less acute, subacute, and long-term radiation-induced injury (RTOG/EORTC scale). These studies, taken together, suggest PTX-vitamin E is most effective when given continuously for months to years after radiation. Furthermore, the recurrence of fibrosis after cessation of treatment suggests PTX-vitamin E disrupts the ongoing processes responsible for facilitating radiation-induced fibrosis; however, it does not permanently irradicate the underlying cause. More work is needed to better define the underlying mechanisms behind the success of this therapy.

12.4 Targeting Chronic Oxidative Stress

Redox changes in tissue can have profound effects on cellular signaling and tissue interactions. In the last decade, superoxide (O_2^-) and other ROS have been shown to play important roles in intracellular signaling and cytokine induction and activation (Bai et al. 1993; Riley 1994; McBride 1995; Schmidt-Ullrich et al. 2000; Delanian et al. 2001; Dhar et al. 2002; Mikkelsen et al. 2003; Cuzzocrea et al. 2004; Moeller et al. 2004). It is becoming increasingly well known that ROS/RNS affect DNA binding and activation of several key redox-sensitive transcription factors, such as SP-1, AP-1, NF-κB, HIF-1α, and NRF1, and growth factors (TGFβ) thought to be involved in radiation-induced normal tissue injury. Experimental studies demonstrate the collapse of antioxidant status, characterized by decreased levels of Cu/Zn-SOD and gluthione peroxides, occurs within hours following radiation (Erkal et al. 2006; Benderitter et al. 2007; Park et al. 2007). The prolonged imbalance between oxygen-derived free radicals and antioxidant capacity following the initial exposure to radiation leads to amplification of signal transduction pathways involved in inflammation and fibrogenesis (Fleckenstein et al. 2007b). Thus, the result is an uncontrolled and progressive increase in oxidative/nitroxidative stress leading to post-translational modification of proteins, changes in transcriptional patterns of genes regulating DNA repair, cell cycle arrest and proliferation, altered cell signaling, release of cytokines and growth factors, and inflammation.

A number of biochemical compounds, such as cysteine, cysteamine, and pentoxifylline/tocopherol, Mn salens, Mn porphyrins, Mn cyclic polyamines, and fullerenes, have been used to target oxygen-derived free radicals in an attempt to reduce radiation-induced damage. Most notably, thiol compounds (amifostine in particular) have been shown in preclinical and clinical settings to reduce normal tissue toxicity from radiation (Brizel et al. 2000; Koukourakis and Yannakakis 2001; Vujaskovic et al. 2002, 2007). Thus far, amifostine has been the only FDA-approved drug for protection against radiation-induced injury in the clinical setting. In the pre-clinical setting, superoxide dismutase-based strategies have been shown to offer the most effective and efficient antioxidant capability. It has been extensively shown that overexpression of SOD or therapeutic delivery of exogenous SOD inhibits radiation-induced changes in a number of biological endpoints, including enzyme activity, membrane integrity, DNA damage, cell transformation, and cell and animal survival (Sanchiz et al. 1996; Epperly et al. 1999; Vozenin-Brotons et al. 2001; Vujaskovic et al. 2002a; Epperly et al. 2003; Khan et al. 2003; Rabbani et al. 2007a; Stinivasan et al. 2007).

The mitigating effect of superoxide dismutases (Cu, Zn-SOD; MnSOD) is the result of its catalytic dismutation of superoxide anion (O_2^-) in a two-step process to oxygen and water. Vujaskovic and colleagues have shown manganese porphyrin mimetics of superoxide dismutase act as pulmonary radioprotectors in vivo as a result of their potent ROS scavenging abilities (Vujaskovic et al. 2002b; Rabbani et al. 2007a, 2007c; Gauter-Fleckenstein et al. 2007). In those studies, long-term administration of MnSOD mimetic improved pulmonary function and reduced morphological and histological damage after radiation (Rabbani et al. 2007a). Most importantly, activation of redox-sensitive transcription factors and signaling molecules in-

volved in inflammation, angiogenesis, and fibrosis, such as TGF-β, HIF-1α, and NFκB, were greatly reduced. In other studies, MnSOD mimetics attenuated levels of macrophage inflammatory protein and interleukin-6 following ischemia/reperfusion injury. In a study by Jackson et al. (2007), TGF-β production by hypoxic macrophages in vitro could be alleviated by incubation with an SOD mimetic, MnTE-2-PyP^{5+}.

In studies by Epperly et al. (1999), a time-dependent progression in pathologic fibrosis after irradiation

Table 12.2. Summary of pre-clinical and clinical studies using target based interventions

	Injury type	Reference
Transforming growth factor-beta (TGFβ) targets	Radiation pneumonitis/fibrosis	Preclinical: Nishioka et al. 2004; Rabbani et al. 2003; Schultze-Mosgau et al. 2003; Anscher et al. 2006, 2008
Recombinant human keratinocyte growth factor (rHuKGF/Palifermin)	Radiation pneumonitis/fibrosis	Preclinical: Chen et al. 2004; Yi et al. 1996
	Esophagitis/nucositis/xerostomia	Preclinical: Dorr et al. 2001, 2005c, 2005b, clinical: Radtke et al. 2005; Brizel et al. 2008
	Gastrointestinal	Preclinical: Farrell et al. 1998
	Bladder	Preclinical: Jaal et al. 2007
Angiotensin-converting enzyme (ACE) inhibitors (butylaminiperindopril, captopril)	Radiation pneumonitis/fibrosis	Preclinical: Ward et al. 1988, 1989; Molteni et al. 2000; Matej et al. 2007
		Preclinical: Moulder et al. 1997a
	Gastrointestinal	Preclinical: Moulder et al. 1993, 1998a, 2007a; Cohen et al. 1997 Clinical: Cohen et al. 2008
	Radiation nephropathy	Kim et al. 2004; Ryu et al. 2007
	Optical neuropathy	
Statins	Radiation induced enteropathy	Preclinical: Haydont et al. 2007c; Wang et al. 2007b Preclinical: Williams et al. 2004).
	Pulmonary fibrosis	
Pentoxifylline ± alpha-tocopherol (vitamin E)	Radiation pneumonitis/fibrosis	Preclinical: Rube et al. 2002; Koh et al. 1995 Clinical: Misirlioglu et al. 2007; Ozturk et al. 2004
	Subcutaneous fibrosis	Clinical: Haddad et al. 2005; Okunieff et al. 2004; Delanian et al. 2003; Lefaix et al. 1999; Delanian 1998
	Uterine	Clinical: Letur-Konirsch et al. 2002
Amifostine	Multiple	Preclinical: Vujaskovic et al. 2002c;
		Clinical: Veerasarn et al. 2006; Antonadou et al. 2003, 2001; Komaki et al. 2004; Koukourakis et al. 2001; Phan et al. 2001; Movsas et al. 2005; Brizel et al. 2000; Trog et al. 1999
		Preclinical: Vujaskovic et al. 2007
Superoxide dismutase-based strategies	Radiation pneumonitis/fibrosis	Preclinical: Gauter-Fleckenstein et al. 2007; Rabbani et al. 2007c, 2007b, 2005; Epperly et al. 2004, 2002a; Guo et al. 2003

indicated increased IL-1 mRNA levels correlated with early radiation pneumonitis, followed by an increase in TGF-β during the development of fibrotic disease and mortality. Moreover, Epperly et al. (2000b) found manganese-SOD plasmid/liposome complex could prevent DNA double-strand breaks, inhibit mitochondrial-dependent apoptosis, reduce vascular adhesion molecule expression (epperly et al. 2002c), and decrease early onset of TGFβ, IL-1, and TNF-α mRNA levels (epperly et al. 2000a), as well as improve median survival time (epperly et al. 2000b). A series of SOD gene therapy studies in animals have also suggested the protective effect of SOD from radiation toxicity in the esophagus and lung (epperly et al. 2000a). Furthermore, it has been shown that the administration of liposomal Cu/Zn-SOD and MnSOD up to 6 months after irradiation in an experimental animal model was shown to reverse radiation-induced fibrosis (lefaix et al. 1996). Studies using a combined treatment of Cu/Zn-SOD and L-NAME was effective against indirect damage caused by reactive species generated in rat lung tissue after radiation (khan et al. 2003). Currently, Cu/Zn-SOD has been used in the clinical application of radiation therapy in Europe to reduce the severity of mucositis, cystitis, and fibrosis (sanchiz et al. 1996; valencia et al. 2002; esco et al. 2004). Cu/Zn-SOD has been shown to reduce DNA damage and chromosomal aberrations, decrease activation of pro-inflammatory transcription factors and signaling molecules, and ameliorate radiation-induced injury (breuer et al. 1992; lefaix et al. 1996; delanian et al. 2001; peter et al. 2001; vozenin-brotons et al. 2001). Multiple studies have shown superoxide dismutase-based strategies reduce expression of pro-inflammatory and profibrogenic cytokines and growth factors, as well as cellular adhesion molecules diminishing leukocyte recruitment into the injured tissue. Furthermore, the elimination of free-radical bioavailability to activate TGF-β results in decreased extracellular matrix formation and increased matrix degradation. The consequence is decreased inflammation and fibro-proliferation and mitigation of architectural/structural damage and overall reduction in lung injury. Table 12.2 outlines a summary of relevant findings.

12.5 Stem Cell Therapy

Recent insight into the role of stem cells in radiation-induced injury has opened the door to new therapeutic interventions focused on mobilization of bone marrow-derived stem cells to replenish the depleted cell population and restore tissue function after radiation. It has been hypothesized that radiation-induced depletion of stem cells in the gastrointestinal villi and salivary glands contribute to the development of acute and long-term injury in these tissues. Several investigators have hypothesized the dose effect of radiation injury was characteristic of the number of stem cells killed after radiation. For example, it has been suggested hyposalivation following radiation for head and neck cancer results from the depletion of the progenitor cell population and inability to regenerate acinar and ductal cells for normal salivary gland function. Lombaert et al. (2006) delivered 15-Gy single-dose irradiation to the salivary glands of female mice transplanted with eGFP+ bone marrow from male mice. The dose was sufficient to induce morphological, histological, and functional injury to the submandibular salivary glands. Salivary glands from irradiated control mice had complete reduction in saliva production at 90 days and substantial atrophy of the glands, decrease in perfusion, and acinar and ductal cell apoptosis at 130 days after irradiation. Bone marrow stem cells from irradiated chimeric mice were stimulated at 10, 30, or 60 days with granulocyte-colony stimulating factor (GCSF). GCSF-stimulated mice showed significantly improved tissue function measured by increased saliva production, reduced salivary gland atrophy, improved gland color and increased gland weight, and restoration of the number of acinar and ductal gland cells. Unexpectedly, neither the acinar nor ductal cells of the irradiated eGFP+ chimeric mice expressed eGFP/Y chromosome upon histological examination, suggesting the increased number of acinar and ductal cells was not bone marrow derived. Based on the proximity of eGFP signal to myoepithelial cells, the authors could not rule out bone marrow origin of myoepithelial cells. However, the presence of co-localized CD31 and eGFP in mesenchymal cells led the authors to conclude that the majority of the bone marrow-derived stem cells in the irradiated tissue were endothelial/mesenchymal and that these cells played a major role in the restoration of gland function and amelioration of histological and morphological injury. Studies by Mouiseddine et al. (2007) support the conclusions by Lombaert's study that stem cells of mesenchymal origin may play a role in amelioration of radiation-induced injury. Mouissedine et al. (2007) found human bone marrow-derived cells migrated to irradiated tissue and tissue outside the irradiated field after local or total body irradiation (francois et al. 2006). These promising studies encourage further exploration

of the potentially beneficial role of stem cell therapy for amelioration of radiation-induced normal tissue injury.

12.6 Additional Targets

The aforementioned therapeutic interventions have been the most well studied; however, as our understanding of the molecular mechanisms of radiation-induced normal tissue injury has grown, novel pathways have emerged that might be targeted to prevent, mitigate, or treat radiation-induced normal tissue. In a recent issue of *Science*, Burdelya and colleagues (2008) used a novel agent, CBLB502, to target Toll-like receptor 5, a ligand for NFκB expressed on enterocytes, dendritic and endothelial cells. Burdelya hypothesized CBLB502, a potent NFκB activator, would prevent NFκB-mediated p53-dependent apoptosis and improve overall survival. In those studies, CBLB502 rescued 87% of mice from lethal total body irradiation in the range of 10–13 Gy. Similarly, Burdelya found compounds that did not activate NFκB in vitro also did not protect mice from lethal total body irradiation. These results, along with studies using TLR5 knockout mice in which CBLB502 was ineffective as a radiation protector, led the authors to conclude that NFκB-mediated activation of TLR5 signaling is necessary for protection against radiation-induced lethality. Molecular analysis of tissue from CBLB502-treated animals demonstrated reduced small intestine toxicity with CBLB502 as compared to irradiated controls, including significantly less apoptosis of cells in the lamina propria and endothelial cells. However, CBLB502 was only effective when given prophylatically, thus limiting its use to therapeutic radiation rather than use for accidental or deliberate exposures, such as in the case of a nuclear accident or attack. At lower radiation doses (<9 Gy), CBLB502 improved overall animal survival (7% controls vs. 40% treated) when given within an hour post-exposure. CBLB502 underwent further evaluation in non-human primate studies. In these studies, CBLB502 did not exhibit any signs of toxicity. Non-human primates received 6.5 Gy total body irradiation ($LD_{50/70}$) 45 min after a single injection of 0.04 mg/kg CBLB502. CBLB502 increased the 40-day survival from 25 to 64%. Likewise, CBLB502 has been shown to have no radioprotective effect on tumors, making this compound an ideal agent for clinical trial.

Other novel pathways for therapeutic intervention have focused on neuroimmune interactions and the use of neuropeptides. The enteric nervous system has in gastrointestinal homeostasis, for example, regulation of secretion, motility, immune function, microcirculation, as well as orchestrates interactions between the immune system and fibroproliferation (Wang et al. 2007c). Thus, Wang, Hauer-Jensen, and colleagues have performed several studies to further explore the role of the neuroimmune system in radiation-induced gastrointestinal injury. In earlier studies, the group found mast cells to be an integral component of the gastrointestinal response to radiation. Mast cell-mediated regulation of epithelial barrier function and vascular permeability, two key components dysregulated in the gastrointestinal syndrome, is under the control of sensory enteric nerves. Thus, the neuroimmune interactions between these cells provide a potential target for therapeutic intervention (Wang et al. 2007a). In a 2006 study, sensory nerve ablation using a neuropeptide increased acute gastrointestinal toxicity (inflammation, mucosal surface area), yet proved effective in reducing fibrosis by a mast-cell dependent mechanism (Wang et al. 2006b). In further studies, Wang and colleagues (2006a) found two neuropeptides, substance P and calcitonin gene-related peptide (CGRP) were increased after localized irradiation of the intestine. Using substance P and CGRP antagonists, the authors found these neuropeptides have opposing effects on the intestinal mucosa during the development of radiation-induced intestinal injury. The authors concluded CGRP antagonists may be beneficial as radioprotectors, and further evaluation is warranted to evaluate their ability to mitigate radiation-induced intestinal injury.

12.7 Conclusions

The identification of molecular pathways involved in radiation-induced normal tissue injury has resulted in the identification of potential candidates for targeted therapies. To date, few of these compounds have been tested in the clinic. The most thoroughly investigated agents for use as mitigators and/or treatment for radiation-induced normal tissue injury have been antioxidant compounds, ACE inhibitors, and growth factors. Interest in this area of research, however, is growing, and newer agents will be developed that may prevent the development of radiation-induced injury in the future. Agents already in the clinic for other purposes, such as the statins, are being tested because of mechanisms of action that are relevant to radiation protection. Thus, in the

near future we should have more to offer patients currently suffering from normal tissue injury from cancer therapy.

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