Treatment of Radiation Injury

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Significance: Radiation exposure as a result of radiation treatment, accident, or terrorism may cause serious problems such as deficiency due to necrosis or loss of function, fibrosis, or intractable ulcers in the tissues and organs. When the skin, bone, oral mucous membrane, guts, or salivary glands are damaged by ionizing radiation, the management and treatment are very lengthy and difficult.

Critical Issues: In severe and irreversible injuries, surgery remains the mainstay of treatment. Several surgical procedures, such as debridement, skin grafting, and local and free-vascularized flaps, are widely used.

Recent Advances: In specific cases of major morbidity or in high-risk patients, a newly developed therapy using a patient's own stem cells is safe and effective. Adipose tissue, normally a rich source of mesenchymal stem cells, which are similar to those from the bone marrow, can be harvested, since the procedure is easy, and abundant tissue can be obtained with minimal invasiveness.

Future Directions: Based on the molecular basis of radiation injuries, several prospective treatments are under development. Single-nucleotide polymorphisms focus on an individual's sensitivity to radiation in radiogenomics, and the pathology of radiation fibrosis or the effect of radiation on wound healing is being studied and will lead to new insight into the treatment of radiation injuries. Protectors and mitigators are being actively investigated in terms of the timing of administration or dose.

SCOPE AND SIGNIFICANCE

DAMAGE TO NORMAL TISSUES remains the most important limiting factor in the treatment of cancer by radiotherapy. Radiation exposure by accident or terrorism may also cause serious problems such as deficiency or loss of function, fibrosis, and intractable ulcers in the tissues and organs. When the skin, bone, oral mucous membrane, digestive tract, or salivary glands are damaged by ionizing radiation, properly managing and treating these injuries can be a long and difficult process. Ionizing radiation causes damage to molecules through energy transmission. This energy generates highly

reactive chemical products such as free ion radicals that can ultimately combine with normal body chemicals and react with cellular components, resulting in intracellular and molecular changes and damage. If lipids and proteins are extensively destroyed, the cellular DNA becomes a primary candidate for the biological and ultimately lethal effects of free radicals by ionizing radicals. Most ionizing radiation-induced damage can be repaired, and enzymatic pathways continue, but radiobiological death may occur when the cells lose their ability to divide and proliferate. Endogenous free radicals, which are collectively named reactive oxygen species (ROS), arise

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Abbreviations and Acronyms

ADSC = adipose-derived stem cell
 ASCO = American Society of Clinical Oncology
 BMMSC = bone marrow-derived stem cell
 CTGF = connective tissue growth factor
 ECM = extracellular matrix
 FGF = fibroblast growth factor
 GWAS = genome-wide association study
 KGF = keratinocyte growth factor
 PCI = percutaneous coronary intervention
 ROS = reactive oxygen species

(continued)

Abbreviations and Acronyms (*continued*)

SNP = single-nucleotide polymorphism

SOD = superoxide dismutase

TD = tolerance dose

TGF β 1 = transforming growth factor beta-1

VSMC = vascular smooth muscle cells

from mitochondrial oxidative metabolism and other reactions in cells.¹ The estimated average generation rate is around 10^9 ROS per cell per day,² which results in 10^6 oxidative DNA damage, 10^5 DNA single-strand breaks, and 0.1 DNA double-strand breaks per cell per day.¹ Additionally, cellular and molecular targets leading to broader issues of signaling and concerns regarding systemic responses to radiation injuries have been investigated, as well as three temporal categories of acute, subacute, and late (chronic), and these are uninterrupted.³ Decreased proliferation, adhesion, and an increased apoptotic cell ratio of fibroblasts occur after whole-body irradiation.⁴ Early signs of epithelial damage include erythema—warmth and tenderness because of transient dilatation of vessels caused by vasoactive amines. The next phase of erythema is caused by vessel damage observed 2–3 weeks after irradiation. The last phase of erythema occurs 10–16 weeks after initial injury and is related to epidermal basal cell death. Appendages such as sweat glands are radioresistant. Erythema may be accompanied by dry desquamation or discoloration. Chronic effects are seen 4–6 months after irradiation. Skin becomes fibrotic with a thin epidermis, which can be easily damaged. Radiation-injured wounds result from the collective effects of fibrosis, necrosis, ulceration, and fistula. Hyper- and hypopigmentation of the skin arise from enhanced activity or damage to melanocytes or the melanocyte–melanosome complex.⁵

CLINICAL RELEVANCE

Radiation injuries solely to skin in interventional procedures have been increasingly reported since the 1990s. The use of fluoroscopic techniques in complicated procedures with a long fluoroscopy time, without proper awareness or dose monitoring, results in a high incidence of these injuries. A

rough estimate of the total number of X-ray procedures that have ever been performed is 3.6 billion procedures, and typically 1% of X-ray procedures are considered angiography and interventional radiology. Thus, some 36 million of these procedures have taken place, and the frequency of major radiation injury is estimated to be between 1:10,000 and 1:100,000 procedures, although the true risk is still unknown.⁶ Thus, 360 to 3,600 cases of major radiation injuries are estimated. In a detailed clinical study of radiation, skin effects occurred from percutaneous coronary intervention (PCI) of the right coronary artery in six chronic total occlusion patients (mild erythema; occurrence rate 1.5%). In most cases, erythema was seen vividly 4 weeks after PCI. A few days after PCI, early erythema can be detected through careful observation. At 7–10 days after PCI, most erythematous pigmentation can be detected. Four weeks after PCI, most skin erythema appears clearly; however, some cases of skin erythema occur without back pain. Subsequently, follow-up every 6 months is needed to detect the reappearance of erythema.⁷ In other infrequent, but socially and psychologically significant, causes of radiation injuries such as direct contact with radioactive materials, accidents at nuclear power plants, and possible terrorism, a greater impact of radiation injury can be observed and the quality of life of patients is severely impaired.

TRANSLATIONAL RELEVANCE

Ionizing radiation is often used for cancer treatment or diagnosis and may be harmful if the dose and duration are not appropriate. Normal tissue toxicity and sensitivity to irradiation are also factors to consider in the development of radiation injuries. As is often seen, local radiation injury occurs due to direct manipulation of high-intensity radioactive sources.

Other causes of radiation injuries include terrorist attack and nuclear power plant accidents. Although the clinical course, severity, and prognosis of local radiation injuries are dependent on the type, source, dose, rate, duration, distribution, location, and size, it is useful to understand the pathogenesis from the cellular to molecular levels for current treatment and future potential.

DISCUSSION

Safety of radiation for the body

Many calculations and studies of the effects of radiation on the human body have been performed in an attempt to maintain patients' safety. Many safety aspects are mentioned in radiation therapy for both external beam radiation therapy and radionuclide therapy. In both procedures, the goal is to deliver a tumoricidal radiation dose to a known tumor while sparing the normal tissue as much as possible. The concept of a minimal and maximal tissue tolerance dose (TD) was introduced and applied to the whole or partial organ volume that received a uniform dose of external beam radiation at as high as >1 Gy/min, with daily fractions of 1.8–2 Gy.⁸ Data were collected of the predicted TD, which was related to a 5% rate of complications within 5 years ($TD_{5/5}$) of the maximum TD, $TD_{50/5}$, which caused a 50% complication rate by 5 years. Depending on the tissue, a 20-Gy single-fraction dose could be biologically comparable to 50 Gy, which is typical for tumor cells, and 10 Gy, which includes some normal tissue. With radionuclide therapy, the tolerance of normal tissues seems greater, but is more variable. Variability is based on differences in dosimetry methodology and in heterogeneous distribution of the radionuclides, which may lead to complications of potential organ toxicity. Measurement and quantification of the heterogeneous radionuclide dose are considered less precise than those of external beam radiation. Heterogeneity of the dose deposition at the cellular level of radionuclides remains a concern.⁹

Effect of radiation on cells, signaling, and the genome

Radiogenomics in prediction of normal tissue toxicity. Radiogenomics aims to develop an assay capable of predicting which radiation-treated patients are most likely to progress after radiotherapy and may personalize and optimize radiation therapy. Single-nucleotide polymorphisms (SNPs) focus on an individual's sensitivity to radiation. An SNP is a DNA sequence alteration targeting a single nucleotide, a point mutation. Considering

the polymorphism, the variant form, a minor allele, has to be present in at least 1% of the population. Even though the frequency of this variant may differ among ethnic groups or geographic regions, the genetic ancestral foundation is largely maintained, or small changes have occurred by natural selection. As human paired homologous chromosomes and each pair of chromosomes carry an allele of the polymorphism, each individual is homozygous for the common allele, and is heterozygous (two of each allele) or minor homozygous for the minor allele. It is estimated that there are 1 million SNPs among 3 billion nucleotide base pairs that cover the human genome. Since haplotype blocks are identical by descent and separated via short recombination hot spots, a genome-wide association study (GWAS), which is necessary for understanding human developmental history, can genotype just a few SNPs (tag SNPs) in a block to estimate nearly all the other SNPs in the same block. In the first GWAS to identify SNPs allied to adverse reactions in patients treated with radiotherapy, SNPs causing erectile dysfunction were found to be most significantly associated, and rs2268363 was located in the *FSHR* gene, the encoded product of which plays a role in male gonad development and function.¹⁰ Since this gene is not related to the radiation response, it might be impossible to detect this SNP with a candidate gene approach. In addition, 4 SNPs found to be related to erectile dysfunction have African ancestry and may not be discovered if searching and investigating only the European ancestry. In GWAS screening of lymphoblastoid cell lines, *BORA*, *MAD2L1*, *PLK4*, *TPD52*, and *DEPDC1B* SNPs were associated with radiation cytotoxicity.¹¹ Even in SNP profiling to estimate the normal tissue response, it is clearly true that other traits and diseases that are likely to increase understanding of genetic variation are associated with radiosensitivity and novel pathways. Whole-genome sequencing and analysis of SNPs associated with the risk of disease have been demonstrated to produce useful and clinically relevant information for individual patients.¹²

Pathology of radiation fibrosis. Deregulated processes after radiation therapy have much in common with the processes of associated diseases that affect the heart, lung, skin, kidney, gastrointestinal tract, and liver. Of the secretory factors driving fibrosis, transforming growth factor beta-1 (TGF β 1), produced by wide-ranging inflammatory, mesenchymal, and epithelial cells, induced fibroblasts and other cells to transform into matrix-producing myofibroblasts. After myofibroblast

activation, collagen production can continue independent of TGF β 1 by autocrine induction of connective tissue growth factor (CTGF). Oxidative stress, hypoxia, and microvascular insults are considered in terms of radiotherapy. Immediate oxidative damage to DNA, protein, and lipid is the primary chemical event. Cell death is a relevant biological response to these early chemical events; however, responses of sublethal damage to cells give rise of fibrogenesis. The direct action of ROS on the extracellular matrix (ECM) is related to this event, because ROS are able to release activated cytokines from extracellular reservoirs.

Microscopically, fibrosis is often focal in distribution and develops an interstitial fibrin network for a long time after radiation exposure. Microvascular injury is also observed. Intimal and media fibrosis causes arterial constriction and contributes to organ fibrosis with atrophy through ischemic hypoxia. Microvascular diminution leads to tissue ischemia, and tissue ischemia develops to fibrosis. Subintimal accumulation of lipid-burdened macrophages is observed in chronic radiation vasculitis, similar to atherosclerosis, where macrophages are a major source of ROS and fibrogenic cytokines. In the radiated human ileum and colon, marked fibrosis of the submucosa, muscularis propria, and subserosa related to the increase of both *CTGF* mRNA and protein after pelvic radiotherapy has been observed.¹³ Deregulated ECM metabolism after radiotherapy is accompanied by the increase of N- and C-terminal collagen peptides assayed in interstitial fluid from patients postradiotherapy.¹⁴

A study using breast skin 1–5 years after 50 Gy in 25 fractions demonstrated a twofold increase of collagen I and III in the early years and increased collagen degradation.¹⁵ Increased ECM degradation and synthesis are specific characteristics of chronic fibrosis. Increased TGF β 1 immunostaining and phosphorylated Smad 2/3 expression are seen in irradiated vascular smooth muscle cells (VSMCs), but not in nonirradiated cells. When human endothelial cells and VSMCs are irradiated, VSMCs stimulate their transition to myofibroblasts.¹⁶ Radiation fibrosis in humans is related to increased collagen synthesis, deteriorated remodeling, and continual activation of fibrogenic growth factors such as TGF β 1 and CTGF. It is difficult to establish an irradiated skin fibroblast cell culture, because irradiated skin in culture has residual DNA damage and radiation-induced differentiation and demonstrates long-term cell cycle arrest. Both postmitotic and mitotic fibroblasts obtained from irradiated skin have contractile and secretory features due to the induction of TGF β 1,

upregulating collagen synthesis and alpha-smooth muscle actin expression.¹⁷ In dermal fibroblasts obtained from eight patients postmastectomy radiotherapy, the differentiation state was significantly different from the preirradiation cells according to the severity of clinical fibrosis.¹⁸ In contrast to radiated skin, primary cell cultures from human intestines demonstrate increased *CTGF* mRNA expression and collagen I protein.^{13,19} Although microvascular injury provides early stimulus of fibrosis and induction of CTGF is dependent on TGF β 1/Smad signaling in normal skin fibroblasts, the active TGF β 1 level in radiation enteropathy remains very low.²⁰ Rho/ROCK (rho kinase) activation may be reversed in the cellular phenotype with inhibition of fibrosis in an experimental rat radiation fibrosis model using a rho/ROCK inhibitor statin, and this is very relevant to clinical phenomena.²¹ As TGF β 1 is one of the most characterized fibrogenic cytokines and can activate myofibroblast progenitors and ECM may be a reservoir of inactive TGF β 1, oxidative leverage is required to release activated TGF β 1. ROS are created by radiation exposure, and TGF β 1 is activated by radiation exposure and upregulated collagen synthesis, which is enhanced by autocrine induction of CTGF. TGF β 1 is also triggered by radiation through a transcription factor, AP-1 family, and also activated by the stress response to DNA damage in skin fibroblasts. A threshold of TGF β 1 activation may exist, and a very early TGF β 1 cascade may be a target of ameliorating radiation-induced late effects.²² High-dose ionizing radiation in rats causes ROS in radiation fibrosis, and ROS release activated TGF β 1 from the ECM and contributes to intracellular signaling molecules in the TGF β 1-dependent pathway.²³ Antioxidant therapy employed after high and single dose of radiation decreases subsequent fibrosis in animals, and the problem remains if these data are related to humans and fractionated radiotherapy.

Effect of radiation on wound healing

Tissue damage by radiation may lead to dermatitis, skin necrosis, and ulcer. In breast cancer patients, an estimated half of the patients underwent radiotherapy. In the course of radiotherapy, over 70% patients experience radiodermatitis, ranging from faint erythema, dry desquamation, moist desquamation, and necrosis. After radiotherapy, patients with radiodermatitis may experience skin fibrosis, which is demonstrated as a change of skin texture, retraction, discomfort, telangiectasia, pain, and itching.²⁴ Grading criteria for radiation dermatitis were introduced by the National Cancer

Institute (NCI) in 2003 and comprise grade 1: faint erythema or dry desquamation; grade 2: moderate to brisk erythema, patchy moist desquamation, mostly confined to skin folds and creases, and moderate edema; grade 3: moist desquamation other than skin folds and creases and bleeding induced by minor trauma or abrasion; grade 4: skin necrosis or ulceration of the full-thickness dermis, spontaneous bleeding from involved sites; and grade 5: death.²⁵ In a randomized phase-III Radiation Therapy Oncology Group (RTOG) study (RTOG 9003), the incidence of grade 3 or worse acute side effects was 35% for conventional fractionation and 54.5%, 50.4%, and 58.8% for hyperfractionation, which divides the frequency of radiation more than usual, split-course-accelerated fractionation, and accelerated fractionation with a concomitant boost, respectively.²⁶ In most patients, radiation dermatitis is mild to moderate (grade 1 and 2), but 20 to 25% of patients experience severe reactions.²⁷ The incidence of severe reactions is dependent on the total radiation dose, dose per fraction, overall treatment time, beam type, energy, and surface area of the skin. In the RTOG 9003 study, the rates of grade 3 and 4 skin reactions were higher in hyperfractionation (11%) and accelerated fractionations with a concomitant boost (11%) than in standardized fractionation (7%).²⁶ In a more recent phase III study, most patients who received over 60 Gy with a concomitant boost regimen, with 53% also receiving chemotherapy, demonstrated mean rates of grades 2, 3, and 4 radiation dermatitis in 54%, 20%, and 4%, respectively.²⁸ In the RTOG 9003, these rates were 49%, 8%, and 0%, respectively.²⁶

Other than skin, radiation injuries are often observed in the oral cavity and intestines. Radiation-induced xerostomia is a common complication of radiotherapy after head and neck cancer. Salivary flow reduces to 50% to 70% of the baseline after 10–16-Gy radiation and is undetectable after 40–42 Gy,²⁹ and radiation-induced salivary gland damage has been investigated. The serum amylase levels rose immediately after radiotherapy, peaked at 48 h, returned to the normal level by day 7, and were undetectable by day 10. Radiation is thought to induce apoptosis of serous cells. Radiation-induced damage to the salivary glands changes the volume and pH consistency of secreted saliva, and secretions become thick and persistent with increased acidity after radiotherapy. In intensity-modulated radiotherapy with partial-volume irradiation, long-term recovery of salivary gland function is reported up to 2 years after completion of radiotherapy.³⁰

Radiation enteritis can be caused by direct and indirect targeting of cellular DNA, leading to impaired cell division or immediate cell death.³¹ The most vulnerable cells to radiation are rapidly replicating cells during the G2 and M phases of mitosis, predisposing to radiation-induced damage, the continually replicating intestinal mucosa, especially stem cells residing in the intestinal crypts. Damage and death of these replicating and differentiating cells may lead to a decrease of differentiated intestinal epithelial cells and loss of mucosal integrity.³¹

Protectors against and mitigators of radiation injury

Agents delivered before or at the time of irradiation with the intent of preventing or reducing damage to normal tissues are known as radioprotectors, while agents supplied at the time of or after irradiation is completed, but before the manifestation of normal tissue toxicity, are called radiomitigators (Fig. 1).

After DNA damage has occurred, many processes take place in the damaged cells, tissue, and organs, including DNA repair activation, signal transduction, expression of radiation-response genes, enhancement of proliferation, and regulation of inflammation. These pathways are important for cell or tissue recovery after radiation exposure, but may also play a pivotal role in the development of tissue toxicity.

Radioprotectors

Free radicals are responsible for continuing a large amount of damage triggered by ionizing radiation. Thus, agents to protect cells from primary free radical damage need to be present at the time of radiation and in sufficient enough concentrations to compete with free radicals produced through radical-scavenging mechanisms. Plenty of radical scavengers and antioxidants exist, which may limit the oxidative stress induced by free radicals. Superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase are examples of naturally occurring antioxidants that defend against free radical-mediated damage; in contrast, the substrates are specific to each enzyme. Generally, the antioxidants used have a low molecular weight, such as hydrogen atom-donating reducing agents, for example ascorbic acid, tocopherols, polyphenols, and thiols such as glutathione. Oxidants are neutralized by hydrogen atom donation, which results in a less- or nonreactive product compared to the original oxidant and a radical product formed by the antioxidant that is no longer detrimental. Scavenging hydroxyl

Figure 1. Radioprotectors and radiomitigators.

radicals, which are formed by radiation-induced damage, can be neutralized by almost all unsaturated organic molecules or molecules that can donate hydrogen atoms. Although hydroxyl radicals can be scavenged with equal efficacy by both radioprotectors and antioxidants, cellular and *in vivo* radioprotection is noted only in radioprotectors. Thus, secondary species generated by hydroxyl radicals are responsible for DNA damage. Thiols such as amifostine and newly developing nitroxides have abundant reactivity to sufficiently scavenge secondary radicals. In contrast, antioxidants such as vitamin C and tocopherol (vitamin E) do not serve as radioprotectors.

Amifostine: a clinically used radioprotector. Sulfhydryl compounds such as cysteine and cysteamine have been known to serve as radioprotectors through free radical scavenging and the donation of hydrogen atoms. Since the first depiction of sulfhydryl/thiol compounds as radioprotectors, more-efficient and less-toxic agents have been developed, such as amifostine. Amifostine is a phosphorothioate that does not enter cells until it is dephosphorylated by alkaline phosphatase.³² When dephosphorylated, the agents freely diffuse into cells and work as free radical scavengers. Amifostine has been proven to disseminate more

rapidly in normal tissues than in tumor tissues.³³ This may be explained by several factors, including tumor blood flow, acidosis of tumors, and lower expression of alkaline phosphatase. Induction of hypoxia via escalated oxygen consumption and DNA concentration may be another mechanism.^{34,35} In many clinical trials, including phase III trials, amifostine has been investigated for the prevention and reduction of acute and late xerostomia, mucositis, dysphagia, dermatitis, pneumonitis, proctitis, and cystitis. Recently, amifostine was tested in randomized trials led by the American Society of Clinical Oncology (ASCO) for the purpose of preventing xerostomia during fractionated radiotherapy, and proved to reduce radiation-induced toxicity.³⁶ In the 2008 ASCO guidelines, amifostine is not supported as a chemotherapy agent targeting advanced head and neck malignancies. Amifostine is recommended for the prevention of mucositis with radiotherapy associated with head and neck malignancies or esophagitis associated with non-small-cell lung cancer. Amifostine toxicity and tumor protection are controversial and have been discussed.³⁷

Nitroxides in clinical development. In addition to amifostine, which is the only clinically available radioprotector, nitroxides are the most promising agents as radiation protectors. Both preclinical

in vivo and *in vitro* studies have demonstrated the oxidized form of a nitroxide as a radioprotector.^{38,39} The leading compound in this class for radioprotection is tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl). A phase I clinical trial in patients receiving whole-bran radiotherapy demonstrated that tempol is effective in preventing radiation-induced alopecia to mild (grade 1 and 2) toxicity, and the systemic level of tempol is negligible after topical application.⁴⁰

Other antioxidants. Antioxidants are radioprotectors, and SOD is used to prevent radiation-induced toxicity. SOD is an enzyme present in human cells which catalyzes the conversion of superoxide to oxygen and hydrogen peroxide and works as an antioxidant after radiation. As SOD is too large to enter cells freely, gene therapy has been used to increase SOD levels in tissues and to prevent or attenuate radiation-induced mucositis in mice⁴¹ or fibrosis in pigs.⁴²

Radioprotectors other than antioxidants. A hormone, melatonin, is considered to act as an antioxidant and increase the expression of antioxidant enzymes such as SOD and glutathione peroxidase. Clinically, melatonin was tested as a radiosensitizer against tumor cells and as a radioprotector for normal cells. In this study, there was no evidence of a longer survival time or better neurologic function than in a historically compared control group.⁴³

Targeting signal transduction pathways have been explored as a mechanism to protect organisms and cells and tissues from ionizing radiation. A polypeptide, CBLB502, delivered before or shortly after radiation protected mice and monkeys from lethal total-body irradiation with less damage to the intestine and bone marrow.⁴⁴ This type of protector is suitable for therapeutic radiation as well as accidental exposure when large areas of the intestine or bone marrow are affected.

Mitigators of radiation

Late-normal tissue toxicity induced by radiation progresses in tissue before the appearance of toxicity. This includes progressive mitotic cell death and eternally active cytokine cascades, which lead to vascular impairment, local tissue hypoxia, and excessive ECM deposition.⁴⁵ The primary aim of radiation mitigators is to stop these cascades or to prevent the continuation of damage, thus reducing the expression of toxicity. In reverse, radiation mitigators can be delivered shortly after exposure to reorganize a critical cell compartment of mucous membranes or bone marrow. In this

situation, mitigators are agents used to avoid acute toxicity. In radiological terrorism, nuclear accidents, and space research, mitigators are a focus in the development of chemopreventatives to decrease carcinogenesis of total-body exposure.

Cytokines as mitigators. Many cytokines and growth factors are actually radiation mitigators if used at or near the time of radiation. They stimulate the differentiation of stem cells in the bone marrow, intestine, or mucosa and inhibit bone marrow failure, gastrointestinal syndrome, or mucositis or xerostomia after total-body exposure. Granulocyte colony-stimulating factor is effectively able to reduce the lethality of total-body radiation exposure by assisting in bone marrow recovery.⁴⁶ Another growth factor, keratinocyte growth factor (KGF), fibroblast growth factor 7 (FGF-7), stimulates many cellular processes, including differentiation, proliferation, DNA repair, and detoxification of ROS.⁴⁷ The characteristics of KGF are associated with the recovery of mucosa after ionizing radiation and prevent radiation-induced xerostomia⁴⁸ and mucositis.⁴⁹ The use of palifermin, a recombinant human KGF, in patients with mucositis after cytotoxic therapy⁵⁰ led to evaluating its use in patients with head and neck cancer who were receiving chemoradiotherapy and experiencing severe and extensive mucositis. Patients receiving hyperfractionated radiotherapy in a phase II study demonstrated a lower incidence of mucositis and shorter duration.⁵¹ Another FGF family cytokine, basic FGF (bFGF) or FGF-2, is primarily found as a potent angiogenic growth factor because of its high capacity for inducing endothelial cell proliferation and migration as well as smooth cell proliferation,⁵² and also accelerates second-degree burn wound healing and improves scar quality.⁵³ In burns, resurfacing with a dermal component is required, and bFGF stimulates wound healing and enhances human skin-derived mesenchymal stem cells under serum-free conditions in a dose-dependent manner.⁵⁴ In a mini-pig receiving 10-Gy external X-ray radiation to the skin and subcutaneous tissue with a tissue expander underneath the subcutaneous tissue, immediate use of bFGF after radiation successfully protected the tissue from subsequent damage by increasing epithelial proliferation, suppressing the induction of apoptotic cells, and enhancing angiogenic cells.⁵⁵

Radiation fibrosis is considered late radiation damage, and TGF β 1 plays a pivotal role in the establishment of radiation fibrosis. For the purpose of neutralizing TGF- β antibodies to prevent lung

fibrosis in rats⁵⁶ or using a small molecule to inhibit TGF β signaling, halofuginone was tested in mice.⁵⁷

Surgical treatment for radiation injury

When conservative therapy such as protectors and mitigators fail to function for late effects, surgery is attempted to remodel and maintain the structures, since irreversible changes and voluntary recovery are not expected. Plastic and reconstructive surgery consists of necrotic tissue removal, i.e., debridement, skin grafting, and local and free-vascularized flaps; in essence, surgical reconstruction, thorough debridement, and anatomically appropriate reconstruction with minor morbidity. This may require multistep surgeries, since the extent and depth of the damaged tissue are obscure.⁵⁸ Radiation wounds may persist for years after radiation with severe pain and skin lesions, limiting range of motion and activities of daily living.⁵⁹ Recent advances in the knowledge and techniques for using somatic progenitor cells or stem cells has promoted less-invasive and more-effective therapy. A mini-pig chronic radiation syndrome model with 50- and 60-Gy cobalt irradiation was similar to humans after a latency period of several weeks and developed skin necrosis over months. Autologous cultured bone marrow-derived stem cells (BMMSCs) applied multiple times enhanced lymphocyte accumulation at the border between the dermis and subdermis and improved vascularization.⁶⁰ In humans, the application of multiple cultured autologous mesenchymal stem cells with skin grafting modulated the radiation inflammatory process by decreasing C-reactive protein.⁵⁸ Two-dimensional gel electrophoretic gel proteomic analysis of the intracellular protein of BMMSCs and adipose-derived stem cells (ADSCs) revealed that the proteins were

Figure 2. Breast cancer, standardized mastectomy (Halsted), and 60-Gy irradiation. Ulcer and scar contracture continued for 35 years. Arrows indicate the scar contractures. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

similar, which suggests that ADSCs can replace BMMSCs in cell therapy.⁶¹ Using noncultured ADSCs, which are 100 times more abundant than BMMSCs per tissue, chronic radiation injuries, including those adjacent to the major organs such as the carotid artery, were effectively treated and showed no recurrence in patients with autologous noncultured adipose-derived regenerative cells, which contain many adipose-derived regenerative cells.⁶² By subcutaneously harvesting autologous adipose-derived cells with a minimal cell-harvesting port and morbidity in patients suffering from chronic radiation wounds and fibrosis for many years after high radiation exposing the bone, tendon, ligament, or cartilage, and demonstrating either necrosis or infection, with meticulous surgical debridement but no additional skin grafting or flap, angiogenic factors such as bFGF and artificial

Figure 3. (A) Meticulous debridement, including necrotic cartilage and bone. (B, C) After debridement, adipose-derived regenerative cells were injected in the edges and bottom of the wound and covered with artificial dermis. The arrows in (C) indicate the loosened tissue fibroses in the axilla and in the parasternal and the peri-wounded areas. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

dermis successfully resulted in complete regeneration and its maintenance for months.^{59,62} As seen in Fig. 2, a 78-year old female patient who underwent a standardized mastectomy and 60-Gy fractionated radiation developed exposed costal rib cartilage and bone in her right chest (Fig. 2). Wound contracture due to fibrosis also occurred (Fig. 2, red arrows). Extensive debridement with autologous adipose-derived regenerative cell therapy with artificial dermis (Fig. 3A, B) healed within 5 weeks, and the scar is more matured and fibrosis is improved (Fig. 3C, black arrows). *Ex vivo* cell culture of adipose-derived cells to investigate their *in vitro* properties demonstrated cell proliferation and differentiation. An ADSC-like appearance emerged in a regular cell culture medium at day 3 (Fig. 4A). Cell growth was uneventful by day 8 (Fig. 4B). These cells are shown in an ES cell culture medium at day 15 at confluence (Fig. 4C), and in the ES cell culture medium at day 15 by differentiation induction (Fig. 4D). In these cells, in ES cell culture medium at day 15, lipid is marked in red by Oil-Red-O staining (Fig. 4E). In this *ex vivo* culture, these cells may be a characteristic of stem cells with the ability to proliferate and differentiate. Therapy with the patient's own abundant adipose-derived regenerative and stem cells with no need of an extensive cell culture facility at a cell processing center can be applied to other surgeries such as for liver

TAKE-HOME MESSAGES

- Development of radiation injuries can be a long-term process.
- The study of radiogenomics for the prediction of normal tissue toxicity shows single-nucleotide polymorphisms (SNPs) in 1% of the population regardless of racial/ethnic background.
- Radioprotectors and radiomitigators can be used before or after radiation exposure to reduce injury.
- Surgery remains the cornerstone of treatment for chronic ulcers and fibrosis.
- Novel therapy using noncultured adipose-derived regenerative cells (including stem cells) is less invasive and has proven effective in elderly patients with meticulous surgical debridement.

disease, type 1 diabetes mellitus, and Crohn's disease,⁶³ and further clinical use may be explored in these organs with radiation injuries.

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Figure 4. *Ex vivo* cultured adipose-derived stem cells, days 3 (A), 8 (B), and 15 (C–E). (D) Induction medium; (E) Oil Red-O. Magnification, $\times 100$. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

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