Introduction

Radiation therapy plays an important role in the management of thoracic tumors. More than 60% of lung cancer patients receive radiotherapy at least once during the course of their disease. Higher radiation doses have been reported to improve local tumor control and patient survival in non–small cell lung cancer (NSCLC). Every 1 Gy above the conventional prescription dose would improve the 3- to 5-year survival rate by approximately 1% and would decrease the hazard from death by 3%.1 The current radiation practice is empirically based on risk estimates for the overall human population to limit the toxicity rate to 5% to 15%.1,3-5 Therefore, the majority of patients do not receive an adequate dose of radiation for tumor control due to concerns of toxicity that occurs in only a small percentage of patients.

Determining the early response of blood markers to ionizing radiation in predicting latent radiation-induced lung toxicity may aid in planning individualized treatment regimens.

The Use of Blood Biomarkers to Predict Radiation Lung Toxicity: A Potential Strategy to Individualize Thoracic Radiation Therapy

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Background: Radiation-induced lung toxicity (RILT) is an important dose-limiting toxicity during thoracic radiotherapy. Early prediction of radiation lung toxicity will allow physicians to determine a customized treatment regimen for each patient and deliver a radiation dose tailored to that individual’s normal tissue sensitivity profile rather than to the average tolerance of the whole population.

Methods: This review focuses on blood biomarkers in predicting radiation-induced lung toxicity. We searched the literature for data associated with cytokines, and we review the updates of proteomic and genetic polymorphisms in radiation lung toxicity.

Results: Studies from single institutions have demonstrated the significant values of cytokines such as TGF-β1, IL-6, KL-6, surfactant proteins, and IL-1ra on predicting RILT. The majority of studies focus on the values prior to and at the end of radiation therapy. There is limited data from proteomics and specific genomic single nucleotide polymorphism studies that target individualized radiation therapy for patients with lung cancer.

Conclusions: Biomarkers or models that can accurately predict radiation-induced lung damage at an early stage, before completion of chemoradiation, would allow physicians to monitor and customize remaining treatment for each patient.
patients who are hypersensitive to radiation. The current radiation dose prescription does not reflect the specific radiation toxicity tolerance of each individual. Stratifying patients according to their risk level for such toxicity and adjusting radiation treatment accordingly would provide a promising tool for individualized radiotherapy. If high-risk patients were excluded, the low-risk patients, who comprise the majority, could receive higher-dose radiation to improve their chance of cure.\(^1\) Early prediction of radiation-induced lung toxicity (RILT) would allow physicians to determine a customized treatment regimen for each individual and deliver a radiation dose tailored to a patient’s normal tissue sensitivity profile rather than to the average radiation tolerance of the whole population.\(^2\)

In general, RILT should include complications from radiation lung damage to the bronchial tree, lung parenchyma, and pleural structure. This paper reviews blood-marker–based predictive models for RILT associated with parenchyma, most often referring to radiation pneumonitis and fibrosis, and we discuss the potential value of biomarkers and comprehensive models to identify individuals at risk for RILT during the early stages of radiation therapy.

### Clinical and Dosimetric Predictors for RILT

Factors such as age, gender, smoking status, location of tumor, and lung dosimetric factors can contribute to the radiation lung toxicity. The most frequently studied are the lung dosimetric factors and the lung dose-volume histogram-based normal tissue complication probability (NTCP) models. Significant factors include point parameters such as volumes (V) receiving a certain dose or higher (such as V30, V20, V13, V5), the dose to a specific portion of the lung volume (such as D30), effective lung volume, and mean lung dose. More extensive reviews on this topic have been summarized by our group and others.\(^3\) In brief, various NTCP models and many dose-volume histogram (DVH) parameters are predictive of the risk of radiation pneumonitis for populations of patients, but none have optimal predictive power (for individuals) for routine clinical use. Furthermore, a statistically significant association or description of complication rates for populations of patients is not equivalent to a good predictor of toxicity for each individual patient. For example, V13, V20, and mean lung dose (MLD) are all predictive of radiation pneumonitis, but they all have a similar suboptimal predictive ability for grade 2 and higher RILT.\(^4\) If cut-

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offs are 30% for V20, 20 Gy for MLD, and 10% for NTCP, these factors have positive predictive values of only 50% to 71% and negative predictive values of 85% to 89%. Fig 1 shows example data from the University of Michigan, indicating that the predictive accuracies of all these dosimetric factors depend on their cutoff values. The cutoff values used in this study generated reasonable (80% to 85%) certainty of identifying low-risk patients but unacceptable (50% to 70%) low-sensitivity and positive-predictive values.

It is believed that many biologic factors determine the intrinsic toxicities of each individual and add to the complexity of toxicity prediction. Individual radiosusceptibility is, in part, genetically determined with a broad variation even after identical dosimetric exposure. For example, about 50% of patients will develop toxicity if the mean lung doses are above 20 Gy. Biological factors may allow us to identify those individuals who will not develop toxicity even if they have already been exposed to high doses to the lung and thus can be treated with higher doses to improve their outcomes.

**Molecular Mechanisms of Radiation Lung Damage: Why Look for Biomarkers in Blood?**

Although the mechanism(s) of radiation lung damage are not yet fully understood, it is critical to review the factors involved in the biologic process of radiation lung damage. Current evidence suggests that many factors and various lung parenchymal cells contribute to the pathogenesis of radiation lung damage. At the molecular level, the initial process may involve direct action of reactive oxygen species (ROS) on DNA, which causes DNA breaks and rapidly triggers the production of cytokines, growth factors, and more ROS, ultimately leading to hypoxia, chronic oxidative stress, and the nonhealing tissue response in the lung. Radiation-induced damage progression is the result of an early activation of an inflammatory reaction leading to the expression and maintenance of an elevated cytokine cascade. Inflammatory and fibrogenic cytokines produced by macrophages, epithelial cells, pneumocytes, and fibroblasts are critical components for modulating and ameliorating the effects of inflammatory reactions after lung irradiation and their sequelae. The molecules involved include (but are not limited to) transforming growth factor beta-1 (TGF-β1), tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), IL-6, and high-molecular-weight mucin-like antigen KL-6. At the cell and tissue level, radiation injures epithelium (such as type I and type II alveolar cells), endothelium, and stromal fibroblasts. The pathogenesis of radiation lung damage is considered to be similar to that of idiopathic interstitial pneumonitis, which is regulated by individual genet-

![Diagram of Molecular Mechanisms of Radiation Lung Damage](image-url)
ic factors and inflammatory phenotypes.\textsuperscript{11} Cells of bone marrow origin were recently found to be involved in drug and radiation-induced pulmonary fibrosis.\textsuperscript{12-15} Other researchers have shown that the collagen-producing lung fibroblasts in pulmonary fibrosis can also be derived from bone marrow progenitor cells.\textsuperscript{12} Phillips et al\textsuperscript{13} reported that systemic circulating multipotent stem cells such as fibrocytes are involved in the process of lung fibrosis. It could be hypothesized (Fig 2) that multiple systems interact within a network of cellular and subcellular signaling and drive the process of radiation-induced lung pathogenesis from each radiation insult. Fractionated radiation therapy as repetitive stimuli could induce initial injuries in lung parenchymal cells. This subsequently causes the release of regulating molecules, which could be known cytokines or molecules yet to be identified. These findings support the notion that blood-borne biomarkers could function as surrogates of early injury in the tissue and thus serve as predictors for the clinical pneumonitis and fibrosis at a later onset. Identifying the molecules specifically associated with radiosusceptibility provides an opportunity to explore reliable biomarkers to predict the risk of radiation-induced lung toxicity.

Typically, radiation damage to lung parenchyma manifests in two phases: radiation pneumonitis and fibrosis. Pneumonitis results from enlargement and atypia of type II pneumocytes, alveolar wall edema, infiltration of inflammatory cells in the interstitium, aggregation of alveolar macrophages, and/or hyaline membranes lining alveolar ducts and alveoli. Fibrosis is an accumulation of fibroblasts and atypical fibroblasts in the interstitium. They both can cause significant reduction in pulmonary function and, in severe cases, cause clinical syndromes. Radiation pneumonitis is perceived to be especially serious as it can be systemically toxic and lethal in the most serious cases. Radiation fibrosis can also cause death but generally only in those who do not have enough pulmonary functional reserve. There are several systems available to grade the toxicity. Unless otherwise specified, RILT in this review refers to grade 2 and above pneumonitis or fibrosis, both of which are equivalent to symptomatic radiation-induced lung injury. These are clinically relevant and have been most frequently studied.

**Cytokine Levels in Predicting RILT**

Cytokines are non-antibody small proteins released by cells that have specific effects on the interactions between cells, on communications between cells, or on the behavior of cells. The cytokines include the lymphokines, interleukins, and cell signal molecules, such as tumor necrosis factor and the interferons, which trigger inflammation and respond to infections. Generally, growth factors would not be classified as cytokines, though TGF is an exception. Cytokines are usually detectable in blood (plasma or serum). Some cytokines act as early surrogate markers for lung damage and have been shown to have a certain correlation with the risk of clinical toxicity. Persistent elevation of cytokines has been proven to mediate the cellular response of normal lung tissue to ionizing radiation.\textsuperscript{13,16} Early cytokine markers would thus aid identification of early surrogate markers that predict the development of late toxicity, thereby accelerating the screening of novel markers for improved treatment. Appropriate inhibition of key cytokines at an early stage might provide a new tool for the effective treatment of RILT. For radiation lung damage, commonly studied cytokines include TGF-\(\beta\), thrombomodulin, interleukins, KL-6, and pulmonary surfactant proteins, reviewed below.

**TGF-\(\beta\)**

TGF-\(\beta\) is activated by ionizing radiation-induced free radicals. It is a prototype of multifunctional regulators of cell growth and differentiation, which stimulate connective tissue formation and decreases collagen degradation resulting in fibrosis. TGF-\(\beta\) plays an important role in both the inhibition of epithelial cell proliferation and the development of tissue fibrosis. Increased local TGF-\(\beta\) expression is accompanied by an elevated plasma concentration. Normal tissue injury is not only mediated by the local production of TGF-\(\beta\) but also influenced by an elevated circulatory level of TGF-\(\beta\) produced by the tumor, which may be taken up and activated at the site of injury.\textsuperscript{9,10,17-19} Many tumors overexpress TGF-\(\beta\) locally and, in addition, most patients whose tumors overexpress TGF-\(\beta\) have elevated plasma TGF-\(\beta\) levels.\textsuperscript{20} The predictive value of TGF-\(\beta\) on lung toxicity was first reported by Anscher et al\textsuperscript{17} in patients with advanced breast cancer treated by high-dose chemotherapy and autologous bone marrow transplantation. The TGF-\(\beta\) levels after induction chemotherapy but before transplantation were significantly higher in patients in whom hepatic veno-occlusive disease or idiopathic interstitial pneumonitis developed than in the controls or the patients without these conditions. The predictive value for the development of either condition was 90% or more when pretransplantation plasma TGF-\(\beta\) levels were more than two standard deviations above the mean established in the controls.

Further study from this same group demonstrated that changes in plasma TGF-\(\beta\) levels may identify patients with lung cancer at high risk for the development of RILT. A persistently elevated TGF-\(\beta\) level at the end of radiotherapy as an independent factor was significantly associated with symptomatic RILT.\textsuperscript{18,19} An elevated pretreatment TGF-\(\beta\) level did not increase the risk of radiation pneumonitis. The plasma TGF-\(\beta\) returning to normal accurately identified patients who would not develop symptomatic pneumonitis. Failure of plasma TGF-\(\beta\) to normalize by the end of treatment,
defined by TGF-β1 levels above two standard deviations of the normal controls, accurately identified patients at risk for symptomatic RILT. Thus, plasma TGF-β1 levels measured at the end of treatment might be useful in selecting patients with normal TGF-β1 levels in whom radiation dose to the tumor can be safely escalated.21-23

Others have reported that TGF-β1 levels in the bronchial alveolar lavage fluid were also predictive of radiation pneumonitis, which is consistent with data from plasma TGF-β1.24 Further study suggests elevation of plasma TGF-β1 levels may be due to an inability of an individual to normally process this cytokine. In many cases, these individuals have lost the mannose 6-phosphate/insulin-like growth factor 2 receptor, which is a key factor associated with TGF-β1 degradation.25

The predictive value of TGF-β1, however, is confounded by the tumor effect, as many lung tumors produce TGF-β1. High TGF-β1 levels during radiotherapy may identify not only patients with a higher risk of developing pulmonary toxicity but also patients with a higher risk of treatment failure.25 Furthermore, the predictive value of the risk of radiation pneumonitis by the presence of increased TGF-β1 levels in the plasma at the end of radiotherapy has not been confirmed by others.26,27 It should also be noted that cytokines are relatively fragile molecules. Technical differences in specimen collection, processing, and laboratory assays also might result in differences in laboratory measurements.28 Should TGF-β1 be validated for RILT prediction, quality assurance of plasma samples are warranted.

Using strict sample quality control, researchers from the University of Michigan recently demonstrated that radiation-induced elevation of circulating TGF-β1 at 4 weeks during the course of radiation therapy was significantly correlated with RILT after completion of radiation therapy in a limited number of patients with NSCLC (Fig 3).29 The TGF-β1 ratio at the end of radiation therapy compared to its pretreatment level was marginally correlated with RILT. There was no significant difference between patients with and without RILT in absolute TGF-β1 levels at pretreatment, at 2 and 4 weeks during, or at the end of radiation therapy. The TGF-β1 ratios (over the pretreatment levels) for patients with and without RILT at 2 and 4 weeks during and at the end of radiation therapy were 2.8 ± 2.2 and 1.0 ± 0.6 (P=.125), 2.3 ± 1.3 and 0.8 ± 0.5 (P=.001), and 1.5 ± 0.9 and 0.8 ± 0.5 (P=.098), respectively. Using 2.0 as a cutoff, the TGF-β1 ratio at 4 weeks during radiation predicted RILT with sensitivity and specificity rates of 66.7% and 95.0%, respectively. The ratio of plasma TGF-β1 level at 4 weeks during over the baseline deserves further study as an earlier predictor for RILT. The RILT prediction during radiation is more useful than the RILT prediction at end of radiation, as the former may provide an opportunity to individualize the remaining treatment and achieve a more successful outcome.

Nevertheless, serial plasma TGF-β1 measurements have been used to identify patients at low risk for normal lung injury from radiation therapy and could thus safely escalate the radiation dose for individualized treatment.30 Using a normal plasma TGF-β1 level after 73.6 Gy of radiation as guidance, Anscher et al31 escalated twice-daily radiation to 86.4 Gy without seeing severe lung toxicity. All grade 4-5 complications occurred in patients whose dose was limited to 73.6 Gy because of a persistently elevated TGF-β1, even though they did not receive high-dose radiation.

**Interleukins**

Interleukins are a group of cytokines (secreted signaling molecules) that are seen to be expressed by white blood cells as a means of communication. Currently, 33 interleukins have been described, which are produced by a wide variety of cells and function at and beyond various levels of the immune system. IL-1 and IL-6 have been commonly studied for their effects on radiation lung toxicity. IL-1, produced by macrophages, induces acute phase reaction and fever. IL-6, produced by macrophages and TH2 cells, is a pleiotropic cytokine regulating many inflammatory and immunologic processes and induces an acute phase reaction to injuries. IL-1α and IL-6 were identified as early circulating cytokine markers for radiation pneumonitis through analysis of a panel of circulating cytokines with different putative functions in radiation pulmonary injury.31 In general, patients with higher baseline levels of inflammatory cytokines are more vulnerable to radiation-induced lung injury.32 Significantly higher pretreatment levels of IL-6 were found in blood.
specimens of patients who went on to develop radiation pneumonitis \((P=.01)\).31

Other researchers have reported that the serum cytokine levels of IL-6 and IL-10 at baseline do not have predictive value for RILT in patients with NSCLC. However, IL-6 levels were significantly higher \((P=.047)\) during the course of radiation therapy in patients with radiation pneumonitis. In the multivariate analysis, covariations of IL-6 and IL-10 levels during the first 2 weeks of radiation therapy were evidenced as independently predictive of radiation pneumonitis in this series \((P=.011)\). A combination of decreased IL-10 and elevated IL-6 blood levels during radiotherapy correlates with the development of radiation pneumonitis.33 Thus, early changes of circulating IL-6 and IL-10 levels during the course of radiation therapy may be used as predictors for the risk of radiation pneumonitis.

**Thrombomodulin**

Thrombomodulin is a transmembrane glycoprotein located on the luminal surface of endothelial cells. Thrombomodulin levels during radiation therapy in patients who do not develop pneumonitis are moderately decreased, while plasma thrombomodulin does not change significantly in patients who develop radiation pneumonitis.34 Patients with lower thrombomodulin levels in the plasma retain more thrombomodulin on the endothelium, thus reducing local hypercoagulability and fibrin deposition.35 The value of thrombomodulin on predicting radiation pneumonitis has yet to be studied.

**KL-6**

KL-6, the lung epithelium-specific protein Krebs von den Lungen-6, is believed to be produced and secreted by type II pneumocytes. KL-6 offers a new perspective as a marker in interstitial lung disease. Serum KL-6 is elevated in a majority of patients with interstitial lung disease and is normal in patients with bacterial pneumonia or in healthy subjects. KL-6 levels depend on the number of regenerating type II epithelial cells and the integrity of the alveolar-capillary membrane. KL-6 is chemotactic for human fibroblasts and may also play a functional role in fibrosis. KL-6 was reported to reflect the severity of radiation pneumonitis, and the increase \((>1.5\text{-fold})\) was associated with serious radiation pneumonitis that was refractory to steroid therapy.36 Blood levels of KL-6 were found to be increased by more than 1.5-fold compared with the pretreatment value in patients who developed radiation pneumonitis. The ratio of blood KL-6 values over baseline at 2 months after the patient had undergone irradiation showed a significant correlation with the occurrence of radiation pneumonitis \((P=.04)\). KL-6 is also a useful marker for prediction of the occurrence of radiation pneumonitis after single, fractional, high-dose stereotactic irradiation of lung tumors.37 In a study of 39 patients treated with fractionated radiation therapy, blood KL-6 levels at 40 Gy of thoracic radiotherapy were found to be elevated significantly in patients who developed radiation pneumonitis compared with patients without radiation pneumonitis.22 Studies with a large number of subjects are needed to validate such results.

**Pulmonary Surfactant Proteins**

Ionizing radiation induces an increased alveolar surfactant that could leak into the blood through radiation-induced endothelial cell damage of the vessel wall. A series of experiments using a rabbit model reported these leaked pulmonary surfactant apoproteins in the serum to be an accurate marker and predictor for later lethal radiation pneumonitis.38 Serum pulmonary surfactant proteins A (SP-A) and D (SP-D) were reported to be useful markers for the early detection of radiation pneumonitis after thoracic irradiation. Serum SP-D levels in patients with radiation pneumonitis were sequentially higher than in patients without radiation pneumonitis. Furthermore, SP-D levels at 50 to 60 Gy (midway during radiation therapy) showed greater sensitivity and positive predictive values for radiation pneumonitis detection (74% and 68%, respectively) than SPA (26% and 21%, respectively). However, another study reported that serum SP-D levels showed no significant difference between patients with and without radiation pneumonitis after thoracic radiotherapy for lung cancer.39 The development of radiation pneumonitis was due to overproduction, not proteolysis of surfactant proteins. With these lung tissue-specific biochemical markers capable of detecting early radiation pneumonitis, more intensive radiotherapeutic strategies would be feasible.40

**Studies of Multiple Cytokines**

A complex cytokine network initiates and sustains the inflammatory and fibrogenic processes associated with radiation-induced lung injury. The ability to simultaneously quantify multiple cytokines would be critical to decipher how they affect RILT. Advances in techniques have made it possible to measure the levels of multiple cytokines in one assay. By using a multiplex suspension bead array system (Bio-Rad Laboratories, Inc. Hercules, California), researchers from Duke University analyzed 17 cytokine arrays in 55 patients before the initiation of radiation therapy to define a cytokine phenotype to predict the risk of symptomatic RILT.41 Although it failed to identify a specific genotype to predict the radiation lung toxicity, this study demonstrated a significantly higher pretreatment level of a neutrophil chemotactic factor IL-8 at baseline in patients without symptomatic RILT. Patients with lower levels of plasma IL-8 before radiation therapy were at an increased risk for developing RILT. Patients who did not develop symptomatic RILT had approximately 4-fold elevated
levels of IL-8 compared with patients who did develop symptomatic RILT. The data demonstrate that patients with increased levels of IL-8 have a decreased risk of developing symptomatic RILT (sensitivity: 68.2%; specificity: 57.6%).

More recently, researchers from the University of Michigan assessed 29 cytokines for their correlation with RILT (F-M.K., unpublished data, 2008). Analysis of matched pairs (6 cases with RILT vs 6 controls without RILT) showed significant differences between the two groups. Fig 4A shows the cytokine changes in a patient with grade 2 and above RILT, which is remarkably different from the matched one without such a condition. Interestingly, the cytokines that generated a radiation damage protective effect, such as IL-1 receptor antagonist (IL-1ra), ratio of IL-1ra to IL-1β (Fig 4B-C) were significantly higher during the course of the treatment for patients who were free of grade 2 and above RILT. The

![Diagram of cytokine changes](image)

**Fig 4.** — (A) Multiple cytokines and radiation RILT. This figure shows changes of 29 cytokines in example patients with and without pneumonitis. (B) IL-1ra and RILT. Patients who developed RILT had significantly decreased level of IL-1ra, while those without toxicity did not change significantly. (C) IL-1ra/IL-1β and RILT. Patients who developed RILT had a significantly decreased ratio of IL-1ra over IL-1β, while those without toxicity did not change significantly.

RILT = radiation-induced lung toxicity, RT = radiation therapy
cytokines stimulating radiation damage (eg, MCP-1, IP-10) often have more significant radiation-induced elevation at 4 weeks during the course of radiation in patients who developed grade 2 and above toxicity at several months after completion of treatment. Future multicenter trials composed of a larger number of cases are needed to study other cytokine markers and to validate these promising findings.

**Proteomics in Radiation-Induced Lung Damage**

As shown in Fig 1, the molecular mechanisms for radiation-induced lung damage involve many factors and various cells through many known and unknown pathways. These factors/cells vary with the stage of the pathologic conditions. In addition to the above biomarkers for RILT, many other protein molecules are closely involved in the early process of radiation damage, and their levels might also have predictive value. The combination of many proteins may be more accurate to predict such toxicity and could therefore be served as a better predictor for clinically significant RILT following treatment. Recent advances in proteomics allow the identification of many other proteins associated with radiation toxicity. Protein profiling techniques allow the rapid comparison of complex samples and the direct investigation of serum or plasma. Consequently, biomarker discovery has been a principal objective in many proteomics studies.

![Proteomics and radiation responses](image)

**Fig 5.** — Proteomics and radiation responses. This figure shows example profiles of SELDI-TOF-MS for C3H and C57 plasma and changes at 2 hours after radiation of both mice strains. A remarkable differential expression is seen at 11.7 kDa between radiated and shammed radiated C57 mice.
To test the feasibility of the proteomic study and search for other markers that might be indicative of radiation damage and toxicity, researchers from the University of Michigan first initiated the potential plasma proteomic biomarkers study with the use of surface enhancing laser desorption ionization-time of flight mass spectroscopy (SELDI-TOF MS) combined with anion exchange chromatography in two strains of mice with various sensitivity to radiation fibrosis (F.M.K., unpublished data, 2005). This study showed significant differences, either upregulated or downregulated, in protein profiles in plasma between radiation-resistant C3H mice and radiation-sensitive C57 mouse well before a histopathologically detectable difference occurred. Fig 5 depicts remarkable differences in proteomic profiles between mice that were sensitive (C57) and resistant (C3H) to radiation pulmonary fibrosis. Examples of differential changes were also seen within C57 mice early after treatment with radiation and sham radiation. Mass spectroscopy-based proteomic techniques may be used to search new markers for radiation lung damage and predictors for RILT.

Using the same SELDI-TOF-MS method, Ménard et al studied changes of pooled serum proteomic profiles in patients with a wide range of cancers before and during radiotherapy in an effort to discover clinical biomarkers of radiation exposure (not severe toxicity). Resultant proteomic profiles were analyzed for unique biomarker signatures using supervised classification techniques. Sixty-eight patients with a wide range of diagnoses and radiation treatment plans provided serum samples both before and during ionizing radiation exposure. Computer-based analyses of the SELDI protein spectra could distinguish unexposed from radiation-exposed patient samples with 91% to 100% sensitivity and 97% to 100% specificity using various classifier models. The method also showed an ability to distinguish high- from low-dose volume levels of radiation exposure with high accuracy. Using direct identity techniques of albumin-bound peptides, known to underpin the SELDI-TOF-MS fingerprints, 23 protein fragments/peptides were uniquely detected in the radiation exposure group, including an IL-6 precursor protein. The composition of proteins in serum seems to change with ionizing radiation exposure. Proteomic analysis for the discovery of clinical biomarkers of radiation exposure warrants further study.

Using a 2-dimensional orthogonal liquid fractionation method, we investigated the proteomic changes in irradiated lung tissues after a single dose of 12 Gy whole-lung irradiation in C3H and C57 mice. Significant alterations were found in at least 15 proteins in fibrosis-resistant (C3H) mice and in 31 proteins in fibrosis-prone (C57) mice compared to untreated controls. These proteins were identified using high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), and they were associated with redox, energy consumption, glycolysis, or chromatin/RNA structure formation. Five of six redox-related proteins including superoxide dismutase (SOD), cytochrome-c oxidase, glutamate dehydrogenase, biliverdin reductase, peroxiredoxin, and carbonyl reductase were downregulated in the irradiated C57 mouse, whereas SOD, sulfotransferase, and carbonyl reductase increased in the irradiated C3H mouse. Decreased antioxidant proteins in the irradiated C57BL/6 mouse may contribute to the extent of lung damage. Expression changes of SOD and 8-hydroxydeoxygenase (8-OHdG), an oxidative stress marker, were confirmed by immunohistochemical staining. These data suggest that a proteomics approach has the potential to generate meaningful information in identifying the early lung responses relevant to latent radiation-induced lung fibrosis.

Applications of such new blood tests may allow personalized proteomic measures of clinical response to radiation therapy and rapid response measures for populations exposed to high doses of radiation. Further refinement of the methods reported in these studies to increase throughput and the range of detection may lead to personalized molecular medicine approaches directly applicable to future clinical trial and patient care, which can be aimed to escalate the radiation dose to tumor while avoiding toxicity for a maximized therapeutic gain.

DNA Repair and Single Nucleotide Polymorphisms of Inflammatory Cytokine Genes

Single nucleotide polymorphism (SNP) is a change in which a single base in the DNA differs from the usual base at that position. SNPs could be traits of certain disease (like sickle cell disease), and other SNPs are normal variations in the genome. SNPs in several specific genes such as LG4, excision repair cross-complementation-2 (ERCC2), and CYP2D6 have been reported to be putative markers to predict individuals at risk for late complications arising from radiation therapy in prostate cancer. The XRCC1 399Gln or APE1 148Glu alleles may be protective against the development of acute side effects after radiotherapy in normal-weight patients with breast cancer.

For patients treated with thoracic radiation, researchers from Beijing recently reported results of SNPs of 20 genes on 236 patients receiving thoracic radiation (with 39 events ≥ grade 2 RILT). A significant association was seen between RILT and polymorphisms at ATM-131717 T/G, ATM-111A/G, and ATM-126713A/G. An increased risk of ≥ grade 2 RILT was associated with the ATM-111 AG + GG genotypes (overall response [OR] = 3.43, 95% confidence interval [CI] = 1.36 to 8.64, P=.006), ATM-126713 AG + AA genotypes (OR = 2.11, 95% CI = 0.979 to 4.529, P=.017), and
Models of Combining Physical and Biological Factors

Certainly RILT involves basic physical dosimetric factors, clinical patient factors, and molecular biologic factors of multiple pathways. An accurate predictive model should consider all of these factors; however, there are limited studies on such an effort. The first study was from Duke University, although it did not intend to generate a more comprehensive model for the prediction. Fu et al stratified the risk of RILT by combined consideration of both dosimetric factors (V30) and biologic factors (TGF-β1). They showed a better identification of patient risk for developing symptomatic RIIT. Fig 6 presents three groups of patients at various risk for RILT. Those with a TGF-β1 level of <7.5 ng/mL at the end of radiation therapy (two standard deviations above the mean of normal controls) and with V30 <30% had a low risk of developing RILT. However, similar work from investigators at the University of Michigan involving a limited number of cases did not show a superiority of comprehensive models over the models of using TGF-β1 level alone. The authors speculated that this was likely due to the fact that majority of their patients received a high dose to the lungs (mean lung dose above 15 Gy).

Conclusions

The evidence has demonstrated a potential value of determining the early response of blood markers to ionizing radiation in predicting latent RILT, which may be used for planning individualized treatment regimens. If proven to be accurate predictors for clinically significant RILT, the potential blood biomarkers or comprehensive models may permit us to select low-risk patients for dose escalation to improve tumor control without increasing radiation toxicity. Safe dose escalation guided by end-treatment TGF-β1 levels suggest that it may also be safe to design dose escalation studies based on biomarkers measured midway during the course of radiation therapy if the latter is proven to be accurate in predicting toxicity in a large series. Additionally, for patients at high risk of RILT, protective measures may be taken to prevent toxicity for the remaining treatment. This group of patients should be excluded for escalation in radiation dose. Such methodology, if used appropriately, will allow individualized radiation therapy for each patient, applying preventive measures for patients at high risk for damage and/or higher dose radiation levels for those at low risk, thus maximizing the therapeutic ratio.

Although no validated blood markers are currently available for daily practice, further research in this area has become important. Due to its radiation-sensitive normal lung tissue and poor outcome under current treatment, lung cancer is a particularly interesting field of study. Blood contains a large part of, if not all, human proteins and is an ideal target for molecular biomarker exploration and discovery due to its ready accessibility.

The future of individualized thoracic radiation is not limited to the issues discussed above. Biological prediction of treatment-related toxicity is more complicated than stated. Clinical patient factors, tumor factors, societal factors, and other interventions (eg, chemotherapy) must be considered in addition to biology/biomarkers and dosimetric factor-guided radiation therapy. Using tumor factors as an example, cytokines other than TGF-β1 involved in treatment toxicity can also be produced by tumors, which will certainly confound the predictive power. We recommend that future efforts regarding individualized radiation should be focused on knowledge-guided radiation therapy, including physical, biological, clinical, and societal considerations. Multicenter trials on this topic are needed not only to validate much of the evidence from single institutions, but also to stratify individuals for their risk for RIIT during the early stages of therapy. This would also provide insight into reliable preventive measures for high-risk patients and suggest guidelines for higher dosage to low-risk patients, ultimately maximizing radiotherapy gains.

Disclosures

No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

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