Radiogenomics: towards a personalized radiation oncology

John D. Roberson II*, Omer L. Burnett III*, and Nathaniel Robin*

Purpose of review
This article evaluates the field of radiogenomics within recent developments in genomics and radiation biology.

Recent findings
Many pediatric cancer survivors have undergone treatment with radiation, putting them at risk for long-term side-effects associated with this therapy, especially cardiac disease and secondary malignancies. Advancements in our understanding of radiation biology have led to the understanding that genetics plays a major role in determining a patient’s susceptibility to developing long-term side-effects, leading to the field of ‘radiogenomics’. Although initial candidate gene studies did not demonstrate replicable genetic variants that affected radiosensitivity, genome-wide association studies have recently begun to identify genes that may help explain some of the observed variation in radiosensitivity. As genomic sciences continues to progress and whole genome studies become more accessible, our understanding of the genes responsible for radiosensitivity will continue to progress.

Summary
The field of radiogenomics continues to evolve with the availability and improved cost of genomic technologies allowing the study of an increasing fraction of the human genome. Studies into genetic factors influencing individual radiosensitivity will increase our understanding of radiobiology and improve our ability to counsel patients on the adverse effects they will likely experience.

Keywords
genome-wide association studies, radiation oncology, secondary malignancies

INTRODUCTION
Radiation therapy is one of the primary means of treating cancer patients. Pediatric cancer survivors – those who were diagnosed prior to the age of 21 years – number nearly half a million in the United States alone and, based on the demographics of the Childhood Cancer Survivorship Study, nearly 60% of them have received radiation [1]. Patients receiving radiation therapy are vulnerable to both early and late side-effects. Given the increasing number of cancer survivors, especially pediatric survivors who have regained a long life expectancy, it is incumbent on both physicians and patients to understand the factors influencing the occurrence, development, and severity of these side-effects. Although radiation biology has laid a solid foundation of the underlying principles guiding why patients develop these toxicities, it has not been able to differentiate why certain patients develop side-effects while others do not. To address this question, radiation oncologists have looked to genomics in an effort to identify those patients more susceptible to these complications. This area is the province of ‘radiogenomics’, a relatively new field that seeks to explain these differences based on genetic variants in the patients. This review, therefore, will focus on evaluating the field of radiogenomics within the recent developments in genomics and radiation biology, documenting its necessity, and describing its methodologies.

*Department of Medicine, †Department of Radiation Oncology and *Department of Genetics, University of Alabama School of Medicine, Birmingham, Alabama, USA
Correspondence to Dr John D. Roberson II, MD, Resident, Department of Medicine, University of Alabama at Birmingham, 1802 8th Avenue S, Birmingham, AL 35233, USA. Tel: +1 205 934 5760; e-mail: jdr25@uab.edu
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KEY POINTS

- Radiogenomics seeks to explain and understand why certain patients develop adverse side-effects while others do not through the identification of genetic variants associated with radiosensitivity.
- Although candidate gene studies initially displayed promising results, their results have yet to be successfully replicated.
- GWAS have begun to produce meaningful results, which will need to be tested among different populations to determine the universality of their application.
- As whole genome sequencing becomes more widespread and researchers are able to assess all the nucleotides within the human genome, there is greater ability to detect genetic variants associated with radiosensitivity.

RADIATION THERAPY

Radiation therapy is a critical component of many treatment regimens for cancer patients. Radiation is a local therapy with minimal systemic effects, like surgery, but does not result in the physical removal of the tumor. Instead, this therapy induces damage to DNA and proteins in cells within the treatment field. This damage occurs both directly and, more importantly, indirectly via the generation of free radicals, which cause single-strand and double-strand breaks in DNA. Cells have several different mechanisms, which allow them to respond to this damage, either by repairing the damage, or in more severe cases, causing themselves to go down the path of apoptosis. Double-strand breaks can be repaired through homologous recombination in which an intact copy of the same chromosome is used as a template to repair the damaged chromosome and nonhomologous end-joining in which chromosomal fragments are rejoined together at ends containing little homology, resulting in regions with nontemplate nucleotides. Single-strand breaks and other DNA damage are often repaired through base excision repair in which glycosylases identify and excise damaged DNA allowing for repair to occur. Other mechanisms exist for repairing individually damaged nucleotides and bulky damage to a single strand of the chromosome [2**,3*].

Because these same repair mechanisms are responsible for guarding the genetic code against mutations, carcinogenesis frequently involves a decrease or loss of a cell’s ability to repair DNA damage, allowing for the accumulation of mutations and the development of a tumor [3*].

Thus, carcinogenesis ultimately results in tumor cells becoming more susceptible to radiation damage through the generation of free radicals than the noncancerous cells. In conventional radiotherapy, the fractionation of radiation doses allows oncologists to take advantage of this differential ability to repair as normal tissue repairs itself in between each radiation fraction more than tumor cells, allowing for the preferential build up of radiation damage and, ultimately, death of cancer cells with a relative sparing of normal tissue.

Yet, even so, radiation may result in significant adverse effects as a result of its damage of normal tissue. Although early adverse effects can result in delays in a patient’s treatment regimen, they are typically transient and patients recover from them soon after completing their treatment course. Contrastingly, late side-effects occur at least 3 months and sometimes even years after the completion of treatment and are responsible for much of the long-term morbidity and mortality associated with radiation therapy [4].

NEED FOR RADIogenomics

Contemporary treatment regimens fortunately have resulted in the long-term survival of about 80% of children diagnosed with cancer [5*]. For example, around 95% of children treated for Hodgkin lymphoma survive 10 years and 85% survive 30 years [6*]. Unfortunately, as contemporary treatment regimens have resulted in longer survival, they have also resulted in an increased risk of developing serious late side-effects. This vulnerability is reflected in the three leading causes of premature death among cancer survivors: cancer recurrence, secondary malignancies, and cardiac disease. Recent studies among Hodgkin lymphoma survivors have reported that the risk of developing secondary malignancies such as thyroid cancer, breast cancer, leukemia, and non-Hodgkin lymphoma approaches 20% or higher within the first 30 years of survival, despite advances in technology which have decreased the size of the radiation field and the doses some patients receive [2**,6*,7*]. Although cardiac damage is more frequently associated with chemotherapies like anthracyclines, radiation therapy can also cause significant damage to the heart. For example, in the Childhood Cancer Survivor Study, about a quarter of those who developed severe coronary artery disease or died of a myocardial infarction by age 45 years had received radiation to their chest. Meanwhile, more than half of those who received radiation to a field including the heart had developed a valvular abnormality by adulthood [5*].
Radiation protocols are designed with the risk of severe late adverse effects in mind such that the risk does not exceed 5–10%. As our understanding of radiation biology has progressed, it has become clear that the 5–10% of most radiosensitive patients are therefore constraining the radiation protocols for all patients, limiting the treatment dose to the more radiotolerant patients receive. Furthermore, while it is true that treatment-related factors affect this radiosensitivity to some degree, it is believed that patient-related factors, primarily genetic in origin, are responsible for about 80% of the variability seen in radiosensitivity [8]. Thus, the more radiotolerant patients may be undertreated in current protocols and could have their radiation dose increased to provide greater tumor control without increasing their risk of developing late adverse effects. Meanwhile, radiosensitive patients may benefit either by receiving different treatment options when available or alternative radiation schedules. And, as it may turn out, their tumors may also be more radiosensitive, requiring less radiation to achieve the same tumor control.

Radiogenomics is the study of the genetics underlying these differences in radiosensitivity. Although radiogenomics will aid in increasing our understanding of the pathways, which mediate the development of adverse events, its ultimate goal is the identification of radiosensitive patients at an increased risk of developing late adverse effects. Meanwhile, radiosensitive patients may benefit either by receiving different treatment options when available or alternative radiation schedules. And, as it may turn out, their tumors may also be more radiosensitive, requiring less radiation to achieve the same tumor control.

**CANDIDATE GENE HYPOTHESIS**

Initially, radiogenomics attempted to study populations who were most at-risk of developing second malignancies, carrying out this study through the candidate gene approach. This initially resulted in the identification of rare high-penetrance genetic variants denoting an increased risk of second malignancies. These genes include those associated with Li–Fraumeni syndrome, retinoblastoma, and neurofibromatosis type 1, and other syndromes involving genes that affect the cellular response to radiation; a genetic assay is the technology most likely to accomplish this [4,9].

![Radiogenomics](https://www.co-pediatrics.com/images/radiogenomics.png)

**FIGURE 1.** Early adverse effects are the result of radiation causing a depletion of cells in the normal tissue (stroma, vascular, parenchymal, and immune cells), allowing for a repopulation of the microenvironment. Late adverse effects result from radiation and cytokines produced by the normal tissue inducing the immune system to produce inflammation and remodeling through profibrotic reactions. Meanwhile, reactive oxygen species produced by the radiation and the immune system cause genomic instability which allows for the clonal expansion of mutations and the generation of a second malignancy. Adapted with permission [10*].
result in increased radiosensitivity through the candidate gene studies [8]. Although further investigation may demonstrate the relevance of some of these SNPs among certain populations, it is clear that these limited number of SNPs are unlikely to provide the desired general explanation for differential radiosensitivity in the population.

GENOME-WIDE ASSOCIATION STUDIES

As technology has progressed and potentiated our ability to develop genomic assays, which are both less expensive and more expansive, radiogenomic research has shifted towards genome-wide association studies (GWAS), permitting simultaneous analysis of a million or more SNPs via a number of different methods [4]. The two-stage approach consists of a first stage in which a discovery group is used to identify SNPs, which may be associated with the development of the phenotype of interest, and a second stage in which the most significant SNPs are analyzed in a replication group using a custom SNP assay (or individual assays). This approach is more cost effective and reduces the multiple-comparisons penalty in the replication stage, allowing for a less stringent P-value to be used to differentiate true positives from false positives. The second approach involves performing a meta-analysis of multiple pre-existing datasets to increase the sample size and statistical power while also controlling for variability within the protocols of individual studies.

Although most GWAS have occurred in adult patients treated with radiation for breast or prostate cancer [7*], one of the most elegant examples of the two-step approach was used to identify SNPs associated with the development of radiation-induced second malignancies in children treated for Hodgkin lymphoma [11]. In this example, the discovery stage consisted of 100 patients who had developed secondary malignancies and 89 patients who had not. Three SNPs were identified which met their adjusted P-value. In their replication stage involving 62 patients who developed secondary malignancies and 71 who did not, two of the three SNPs (rs4946728 and rs1040411) were still significant. Both SNPs were located on chromosome 6q21 intergenic between ATG5 and PRDM1. Patients with both risk alleles expressed less PRDM1 mRNA, and while cells with both protective alleles had an increase in PRDM1 mRNA following exposure to ionizing radiation, these cells did not. Furthermore, since PRDM1 negatively regulates the proproliferative gene MYC, cells with the protective alleles had significantly more repressed levels of MYC. Interestingly, they found no association between these same SNPs and the development of radiation-induced second malignancies in adults treated for Hodgkin lymphoma. This suggests that the age of exposure to ionizing radiation modifies the association between these SNPs and the development of secondary malignancies. Even so, it will still be important to follow-up these results in other cohorts. Finally, this study also serves to demonstrate that many SNPs that have been identified in these studies are intergenic or occur in genes previously not known to affect the phenotype under investigation, meaning that more research will be required to determine the mechanism by which these SNPs affect the development of adverse effects.

One of the major challenges faced by GWAS involves recruiting enough patients on whom to perform these studies in order to correct P-values for multiple comparisons. This problem is only compounded by the necessity of needing these patients to have comparable pre-treatment and post-treatment symptoms and to have received treatment regimens whose variability does not modify the effects substantially [2**]. Furthermore, much like the effect of age on the association between the variants in 6q21 and the development of secondary malignancies, research in adult patients has also demonstrated a surprising amount of interethnic variability in the relationship of SNPs and late radiation side-effects – even as some SNPs demonstrated a positive association among one ethnic and a negative association in another [9]. It is also important to recognize that these late effects often do not appear until 5 or more years after the completion of treatment, underscoring the importance of consistent, granular, and long-term follow-up if these relationships are to be discovered. In order to ameliorate these challenges, the Radiogenomics Consortium was founded in November 2009 and currently has over 190 members at 115 institutions across 26 countries. The members of this Consortium collaborate on projects to identify SNPs associated with radiation-induced adverse effects through sharing data and performing meta-analyses [12*].

CONCLUSION

Radiogenomics fulfills a needed role within radiation oncology – explaining and seeking to understand why certain patients develop adverse side-effects, especially life-threatening side-effects, while others do not. After the disappointing inability to replicate candidate gene studies and the recent development of GWAS, this field remains within its infancy. Although GWAS have begun to produce meaningful results, these results still need to be tested among different populations both for confirmation and to determine how universal their...
application will be. A final consideration is the recognition that radiosensitivity is unlikely to be an all-or-nothing property of an individual’s genes – it is anticipated that predictive assays will identify some patients as being radiosensitive for one adverse event while remaining radioresistant to another. This increased complexity of their individual side-effect profile will result in an increasing need for physicians and other healthcare providers to serve as counselors, helping them weigh the different aspects of their side-effect profiles in a probabilistic analysis within the context of their potentially life-threatening malignancies. In the future, much like candidate gene studies were replaced with GWAS studies, GWAS studies that assess SNPs in the order of millions will be replaced with whole genome sequencing allowing researchers to assess the billions of nucleotides in the human genome.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING
Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest
■■ of outstanding interest


3. The author of this review provides a brief overview of the developing knowledge of the role of genomic variation in the development of therapy-related secondary malignant neoplasms and the methodological challenges to increasing our understanding of the molecular underpinnings of their development.


This article provides a straightforward discussion of the mechanisms underlying DNA damage and repair as related to tumorigenesis.


This article provides detailed epidemiological data regarding radiation-induced cardiac disease among both pediatric and adult cancer survivors.


This article discusses the risk of developing secondary malignancies in pediatric patients treated with radiation therapy in Germany, Austria, and Switzerland. Also, this article provides details regarding the evolution of treatment regimens for Hodgkin’s lymphoma.


The authors review the association between secondary malignant neoplasms and several different primary cancer, including pediatric tumors. They also discuss nongenetic factors which may influence this association, including age, hormones, chemotherapy, and type of radiation treatment.


This article reviews the different systems proposed to be involved in the generation of side-effects from radiotherapy, including early and late adverse side-effects as well as secondary malignancies.


Overview of the Radiogenomics Consortium including a list of radiogenomics and radiobiology publications developed from their collaborative work, mostly on adults.