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Radiogenomic Predictors of Adverse Effects following Charged Particle Therapy

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Abstract

Radiogenomics is the study of genomic factors that are associated with response to radiation therapy. In recent years, progress has been made toward identifying genetic risk factors linked with late radiation-induced adverse effects. These advances have been underpinned by the establishment of an international Radiogenomics Consortium with collaborative studies that expand cohort sizes to increase statistical power and efforts to improve methodologic approaches for radiogenomic research. Published studies have predominantly reported the results of research involving patients treated with photons using external beam radiation therapy. These studies demonstrate our ability to pool international cohorts to identify common single nucleotide polymorphisms associated with risk for developing normal tissue toxicities. Progress has also been achieved toward the discovery of genetic variants associated with radiation therapy–related subsequent malignancies. With the increasing use of charged particle therapy (CPT), there is a need to establish cohorts for patients treated with these advanced technology forms of radiation therapy and to create biorepositories with linked clinical data. While some genetic variants are likely to impact toxicity and second malignancy risks for both photons and charged particles, it is plausible that others may be specific to the radiation modality due to differences in their biological effects, including the complexity of DNA damage produced. In recognition that the formation of patient cohorts treated with CPT for radiogenomic studies is a high priority, efforts are underway to establish collaborations involving institutions treating cancer patients with protons and/or carbon ions as well as consortia, including the Proton Collaborative Group, the Particle Therapy Cooperative Group, and the Pediatric Proton Consortium Registry. These important radiogenomic CPT initiatives need to be expanded internationally to build on experience gained from the Radiogenomics Consortium and epidemiologists investigating normal tissue toxicities and second cancer risk.

Keywords: radiogenomics; single nucleotide polymorphisms; normal tissue toxicities; second malignancies

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Introduction

The goal of radiogenomics is to identify genomic factors that are associated with the clinical variability observed in response to radiation therapy [1, 2]. A principal aim of research in this field is discovering and validating biomarkers predicting susceptibility to develop adverse effects resulting from exposure to the high doses of radiation used to treat cancer. One hypothesis that underlies research in radiogenomics is that germline genotypic variation accounts for a portion of the variance observed in radiation toxicity. In this regard, it should be noted that the development of adverse events following cancer treatment with radiation continues to be of importance despite significant technical advances in radiation therapy to conform the dose of radiation to the tumor. Nevertheless, moderate (grade 2) reactions still develop in approximately 10% to 20% and severe (grade 3) complications in 2% to 5% of cancer patients treated with radiation [3].

Evidence that the variation in response to radiation may have, at least in part, a genetic basis, comes from the existence of several rare genetic syndromes associated with hypersensitivity to radiation [4]. For example, mutations in *ATM* result in ataxia-telangiectasia, a syndrome characterized by extreme radiosensitivity and increased risk for developing cancer. Similarly, rare mutations in other genes that play a central role in DNA repair such as *NBS1* (Nijmegen breakage syndrome), *MRE11* (ataxia telangiectasia-like disorder), and *LIG4* (DNA ligase IV deficiency) also lead to syndromes that are characterized by severe radiosensitivity.

An important aim of radiogenomics is the development of assays based on validated genomic biomarkers that predict outcomes following radiation therapy. The creation of such genomic tests should help practitioners select the most appropriate treatment for each patient on an individual basis and enhance the ability to perform precision radiation therapy. In vitro studies of irradiated cells have estimated the heritability of radiosensitivity to range from 58% to 82% [5–10]. In addition, results of clinical studies have demonstrated that patient-related characteristics, including inherited genetic factors, could represent an important basis influencing susceptibility to develop radiation-related toxicities [11]. Substantial progress has been achieved toward the discovery and validation of these genomic biomarkers, primarily single nucleotide polymorphisms (SNPs) [2]. However, only a limited number of SNPs have been validated in multiple cohorts and relatively few mechanistic studies have been conducted. A major focus of this research has been the identification of SNPs associated with adverse effects following prostate, breast, or lung cancer radiation therapy, although new cohorts treated for head and neck, brain, or rectal cancers are being established for radiogenomic research. A critical factor that has helped to advance work in this field was creation of the Radiogenomics Consortium (RGC) [12], a National Cancer Institute–supported cancer epidemiology consortium that currently consists of 232 member investigators at 123 institutions in 32 countries [13]. The common aim of the RGC membership is to share biospecimens and data so as to achieve large-scale studies with increased statistical power to enable identification of relevant genomic biomarkers. However, in order to achieve successful clinical translation of this research, discovery studies must be large enough to identify SNPs associated with an increased risk for the development of adverse effects, results from these discovery studies need to be replicated in independent populations, and clinical studies are required to evaluate the risks and benefits associated with changing a treatment plan based on the validated biomarkers.

Importance of Radiogenomic Research for Patients Treated with Charged Particle Therapy (CPT)

A fundamental rationale for the use of CPT is that the dose can be better conformed to the tumor because it is delivered preferentially in the region of the Bragg peak with a lower integral dose to normal tissues and organs at risk [14]. However, a reduction in toxicity has not always been observed in comparison with photon treatment. In particular, a concern has arisen related to brain stem necrosis following proton irradiation of brain tumors in pediatric patients [15–18]. It is therefore clearly of interest to perform radiogenomic studies using patient cohorts treated with CPT. Most published radiogenomic studies have involved cohorts irradiated with photons, and there are none in patients who received CPT despite increasing numbers of patients with cancer being treated with protons or carbon ions. However, efforts are underway to establish cohorts comprising patients undergoing protocols using CPT for radiogenomic studies. It should be noted that although electrons represent a form of charged particle, this article focuses primarily on carbon ions and protons since these are the principal forms of CPT that are currently being developed and coming into more widespread use in the clinic.

The rationale for ensuring that radiogenomic research includes CPT cohorts is that the genomic markers identified from studies of patients treated with photons may not be entirely applicable as the radiobiology could differ between the 2 modalities. That is, it may be more than just a matter of dosimetry and sparing of normal tissue that distinguishes CPT from photon radiation therapy as the nature of the biological processes occurring in cells and tissues following photon versus

charged particle irradiation may differ [19]. Although there will likely be genetic variants important for both types of radiation, it is possible that some might be specific for the different forms of radiation because of differences in their biological effects. In support of this hypothesis are the results of cellular radiosensitivity studies suggesting that variations in proton relative biological effectiveness (RBE) may be partly due to defects in the Fanconi anemia/BRCA DNA repair pathway [20].

Carbon ions have a high linear energy transfer (LET) and produce complex DNA damage that is difficult to repair [21]. Although protons over much of the spread-out Bragg peak (SOBP) have a lower LET compared with carbon ions, the DNA double-strand breaks they produce are more complex compared with photons [22, 23]. It has even been suggested that protons induce more DNA damage than heavier ions at the same LET [24]. In addition, evidence indicates that altered patterns of repair occur, including a preference for homologous recombination in cells irradiated with protons and greater sensitivity when it is defective [25, 26]. Other differences in biological effects include production of reactive oxygen species [27], complex chromosomal alterations [28], micronuclei, and apoptosis [29]. Epigenetic changes also appear to differ as photons have been reported to cause DNA hypomethylation while protons primarily induce DNA hyper-methylation [30, 31]. In addition, cells irradiated with protons exhibit altered migration and invasion compared with photons [31, 32]. The results of these studies suggest that important biological differences occur following irradiation with charged particles compared with photons that could result in an altered range of genomic factors associated with outcomes from these different forms of radiation. Although many of the differences observed for protons compared with photons could be a reflection of the higher LET values toward the distal edge of the SOBP, results suggest that some of these differences may also occur in the lower LET portions of the SOBP.

In order to address the lack of radiogenomic research for patients treated with CPT [33], a collaboration was established between RGC investigators and the Proton Collaborative Group (PCG). The PCG provides proton therapy–specific clinical research oversight to 9 independent treatment centers and institutional members across the United States. The PCG administers 5 clinical trials in the areas of breast, prostate, and lung cancer and maintains a registry (NCT01255748) that collects prospective data for patients treated with protons. In addition, this consortium tracks the outcomes over time for these tumor sites as well as pediatric cancers. The project titled "Evaluation Tracking Project: A Prospective Chart Review of Patients Treated with Proton Therapy" is a prospective observational study with the goal of enrolling more than 20 000 patients. It was designed to collect and analyze patient information to evaluate the disease process and proton treatment–related outcomes with the goal of improving patient care [34]. The study began in June 2009 with a target follow-up duration of 30 years. Demographic and clinicopathological variables, such as age, race, diagnosis information (Gleason Score, prostate-specific antigen at different time points, and stage), prior treatment and medication information, type of proton protocol, dosimetry, follow-up, toxicity, and quality-of-life data at different time points are included in the PCG database. This registry has been used for research in proton dosimetry, and studies have compared treatment-planning strategies. Efforts have been initiated to create a biorepository comprising biospecimens from patients whose clinical data have been entered into the PCG database. This biorepository/databank will serve as the basis for radiogenomic studies for patients treated with protons. In addition, collaboration of the RGC with investigators associated with the Pediatric Proton Consortium Registry and the Particle Therapy Cooperative Group [35] is also a high priority in order to expand patient cohorts treated with charged particles for radiogenomic studies. It should also be noted that DNA samples have been genotyped from approximately 200 men treated for prostate cancer with carbon ions at the Japanese National Institute of Radiological Sciences with the results being included in future radiogenomic studies. It is anticipated that as additional centers with proton and carbon ion capability are completed where RGC investigators are located, that the size of patient cohorts receiving CPT will increase.

Subsequent Malignancies following Radiotherapy

As most previous reviews of radiogenomics have focused on normal tissue toxicities [2], a critical area that has not received adequate attention for radiogenomic studies has been the identification of genomic biomarkers associated with the development of a new cancer following radiation therapy. Studies in this area are difficult because of the long time scale for radiation-induced malignancies to appear following the initial irradiation and the need for long-term longitudinal studies. It should be noted that this area of research is particularly pertinent to CPT because there is substantial uncertainly as to the radiobiology of charged particles compared with photon irradiation in regard to cancer induction. In addition, there is some concern about the whole-body scatter doses from neutrons, which are more carcinogenic than photons, and associated with passive scattering delivery systems used for some proton irradiations [36, 37].

Clearly, a subsequent malignancy is one of the most serious late adverse effects of radiation therapy, causing substantial morbidity and mortality in cancer survivors. Over the past 40 years, numerous epidemiologic studies provided insight into

radiation therapy–related risks for a variety of tissues [38–42]. The highest risks (>5-fold increase) were reported for subsequent malignancies of the brain, bone, soft tissue, skin (basal cell carcinoma), breast, and thyroid [43]. More modestly elevated risks were also observed for subsequent malignancies of the esophagus, stomach, colon, rectum, anus, bladder, pancreas, lung, salivary gland, and hematopoietic system. For nearly all tissues, risks generally become evident >5 to 10 years following exposure, but then they persist for decades and appear to increase with increasing radiation dose without evidence of plateau or downturn. Exceptions to these observations include the thyroid, for which a decrease in risk was observed for doses greater than 20 Gy, and the hematopoietic system, for which increased risks are observed within several years following exposure. Childhood cancer survivors consistently have the highest relative risk of developing subsequent malignancies, though significantly elevated risks typically persist in patients treated with radiation therapy as adults, in whom absolute risks generally are higher.

Beyond age at exposure, other factors may also modify radiation therapy–related risks for subsequent malignancies. Patients frequently receive systemic therapy in addition to radiation (either as part of a combined modality protocol or sequentially to treat relapsed/refractory disease), and increasing attention has been paid to the joint effects of systemic therapy and radiation therapy on subsequent malignancy risks. Patients who received cytotoxic chemotherapy and radiation therapy have a higher risk of developing malignancies of the lung, pancreas, gastrointestinal tract, bone, and soft tissue compared with those receiving radiation therapy alone [44–47]. In particular, a striking supra-additive effect between abdominal radiation therapy and receipt of the oral alkylating agent procarbazine on stomach and colorectal cancer risk was observed among survivors of Hodgkin lymphoma [47, 48]. Beyond cytotoxic therapies, anthracyclines have also been associated with subsequent malignancy risks [49], a particularly intriguing observation given that doxorubicin is a commonly implicated drug in radiation recall [50]. In contrast to other outcomes, radiation therapy–related risks for breast cancer after Hodgkin lymphoma and childhood cancer appear lower among survivors who received gonadotoxic systemic therapy or pelvic irradiation, likely due to poor ovarian function [51]. Limited research has focused on the potential for lifestyle factors to influence radiation therapy–related risks for subsequent malignancies [40]. Among these, perhaps the most important is the suggestion that cigarette smoking and chest radiation therapy may have a multiplicative effect on risk for subsequent lung cancer after Hodgkin lymphoma [45].

Understanding of radiation therapy–related subsequent malignancy risks to date is based almost exclusively on patients treated with external beam photon therapy. Major advances in radiation therapy, particularly in the past 2 decades, have substantially changed dose distributions, often resulting in reduction of normal tissue dose, which should theoretically improve outcomes among patients [52, 53]. However, the actual impacts of these changes are poorly understood due to small sample sizes and insufficient duration of follow-up. These challenges are further accentuated for studies of subsequent malignancies after CPT because expanded use of protons and carbon ions has been more recent. Exemplifying these challenges, the first study of subsequent malignancies after proton therapy compared 558 patients treated with protons from a single institution with the same number of patients who received photons using data taken from a US population–based cancer registry [54]. With a median duration of follow-up of ~6 years, that study reported a lower risk of subsequent malignancy in the proton compared with the photon cohort. However, detailed treatment data were lacking, follow-up approaches differed between the comparison groups, and the differences in subsequent malignancy occurred primarily in the years immediately after treatment and thus were unlikely to be attributable to radiation therapy [55]. Fortunately, the international research community recognizes both the challenges and importance of this research area and has launched a collaborative effort to investigate subsequent malignancy risks in pediatric cancer survivors treated with protons [33].

Germline Genetic Variation and Risk of Radiation Therapy–Related Subsequent Malignancies

Whereas all radiogenomic studies are hampered by the challenges of small sample sizes and lack of available study populations with both detailed clinical data and available DNA, studies of subsequent malignancies face the additional hurdle of requiring long-term follow-up because many second cancer diagnoses occur decades after exposure. Because of the relative paucity of available information, our current understanding of germline genetic variation and risk of radiation therapy–related subsequent malignancies lags far behind our understanding of genetic factors associated with cancer predisposition. In addition, no study has investigated the potential for germline genetic variation to modify subsequent malignancy risks after CPT.

Inherited predisposition to cancer has long been recognized through the occurrence of multiple primary malignancies in families [56, 57]. In the general population, most studies of genetic modifiers of radiation therapy–related risks have focused on genetic variation in DNA damage detection and repair mechanisms [58]. Among adults, the most comprehensive study to date was the Women's Environmental Cancer and Radiation Epidemiology (WECARE) Study, a multicenter US-based case-control study of contralateral breast cancer designed to investigate the joint effects of radiation therapy, systemic therapies, genetic susceptibility, and other lifestyle and medical history factors on breast cancer risk [59–61]. Among the women in the WECARE study, the polygenic risk score, which was calculated by summing the number of risk alleles for each SNP, was associated with breast cancer risk. Risk alleles represent those loci that had been determined in previous studies as being associated with an increased breast cancer risk [62]. This risk difference was accentuated among women diagnosed with breast cancer at a younger age or whose contralateral breast cancer occurred $\geq$5 years after first primary breast cancer. Another study exemplifying previous candidate gene research evaluated a cohort of survivors of head and neck squamous cell carcinoma [63]. That study observed elevated subsequent malignancy risk for high-risk versus low-risk genotypes in *TP53* and *TP73* SNPs among individuals who had received radiation therapy and/or chemotherapy but not among those who had received surgery alone.

While the specific findings described earlier are intriguing, overall the results from these candidate gene studies investigating genetic susceptibility to treatment-related subsequent malignancies have been disappointingly inconsistent. Likely contributors to the inconsistent results include variability in study design (eg, different patient populations and definitions of outcomes), limited sample size, lack of detailed treatment information, combined modality protocols involving the use of systemic treatment in addition to radiation therapy, and lack of replication of the reported findings in an independent population.

More recently, genome-wide association studies (GWAS), which agnostically interrogate common variation across the genome, have been used to identify novel susceptibility loci for subsequent malignancy risk. The first included 80 patients with therapy-related myelodysplastic syndrome/acute myeloid leukemia and 150 cancer-free controls, with replication of their results in an independent population [64]. That study identified suggestive associations for 3 SNPs: rs1394384 intronic to *ACCN1*; rs1199098 in linkage disequilibrium with *IPMK*; and rs1381392, which is not near any known genes. However, the analyses lacked detailed treatment data. Another study investigated risk of all subsequent neoplasms after Hodgkin lymphoma [65] from the Childhood Cancer Survivor Study (CCSS) who were treated with radiation therapy (96 cases, 82 controls) as well as a replication set of cases and controls identified from high-risk cancer clinics. That study found significant associations for several variants on chromosome 6q21 that were correlated with expression of *PRDM1*, a zinc finger transcriptional repressor, and radiation-induced *MYC* repression. However, again, detailed treatment data were not used in the analysis.

The most recent GWAS expanded the study of genetic susceptibility to subsequent malignancies, combining data from CCSS with the St Jude Lifetime cohort [66]. Both of these childhood cancer survivor cohorts have the rare combination of available DNA, detailed treatment data, and long-term systematic follow-up for the occurrence of subsequent malignancies in a large study population. Additionally, all survivors in both cohorts with available DNA have been genotyped, rather than just selected subsets as in the previous nested case-control studies. The first study investigated breast cancer risk after childhood cancer (207 cases, 2774 controls). Radiation dose to the breast was reconstructed for all survivors in both cohorts based on individual patient radiation therapy treatment data, assuming standardized field configurations. Associations were identified for loci at 1q41 (rs4342822, nearest gene *PROX1*) and 11q23 (rs74949440, nearest gene *TAGLN*) that were restricted to survivors who received $\geq$10 Gy radiation exposure to the breast. In contrast, another association was identified at 1q32.3 (rs17020562, nearest gene *RPS6KC1*) only for survivors who had received $<$10 Gy radiation exposure to the breast. Risk estimates were consistent in both the CCSS and St Jude Lifetime cohort populations.

Overall, these GWAS support the hypothesis that germline genetic susceptibility could modify radiation therapy–related risk for subsequent malignancy. However, limitations in sample size still remain; thus, findings require replication in independent populations. Heterogeneity within the survivor cohorts (eg, by first primary type, age at exposure) and outcome definitions (eg, by combining all subsequent malignancies together) may further hamper generalizability of the findings. Importantly, the risks associated with variants in each of the GWAS described earlier were generally higher than those in most GWAS of first primary sporadic cancers, with per-allele risk estimates typically $\sim$2-fold or greater for subsequent malignancies compared with 1.1 to 1.2 for most sporadic cancers, except for certain types, such as testicular cancer and acute lymphoblastic leukemia/lymphoma [67, 68]. It remains to be seen if these higher effect sizes will persist as the findings are replicated in additional independent populations, or whether they represent a so-called winner's curse, whereby the effect sizes are overestimated in the initial

discovery studies. Accurate quantification of gene and radiation therapy joint effects will be essential for translating these findings into the clinical setting.

It should also be noted that certain cancer susceptibility syndromes are considered to increase the risk of radiation-induced cancers. For example, preclinical data show that *NF1* loss in mice is associated with diverse radiation-induced tumors [69]. Evidence in humans is substantially more limited due to small sample sizes. While case reports and small series suggest that individuals with Li-Fraumeni syndrome may have an elevated risk for second malignancies following radiation therapy [70, 71], larger-scale studies with detailed data on radiation exposure and genetic mutations are needed to clarify whether these risks truly are substantially higher than expected (ie, supra-additive) [72–74].

Radiation Dose Assessment

The need for accurate dosimetric information represents a critical challenge for radiogenomic studies because dose clearly affects the probability for the development of adverse responses to radiation therapy. The gold-standard study design uses detailed, dosimetric radiation therapy treatment data to estimate absorbed dose for organs in the radiation field or those receiving a significant scattered dose [75]. However, for retrospective studies, uncertainties in dose reconstruction following radiation therapy often arise due to a lack of detailed dosimetric data. This is a particularly large challenge for CPT due to multiple uncertainties when attempting to retrospectively recreate dose distributions [76]. As we move to study the potential for germline genetic variation to modify radiation therapy–related risks, accurate dose assessment is increasingly important. Previous studies have clearly demonstrated that misclassifying an environmental factor will bias estimates of gene-environment interactions, increasing the sample size required to detect associations [77, 78]. In addition, there is substantial uncertainty in the RBE values for charged particles, particularly protons that are conventionally assumed to have an RBE of 1.1. However, it may be higher, particularly in the distal edge of the SOBP. A notable example of a radiogenomic study that has made a substantial effort to obtain comprehensive dose information is REQUITE (validating predictive models and biomarkers of radiation therapy toxicity to reduce side effects and improve quality of life in cancer survivors), for which a series of dosimetric parameters, including the full dose-volume histograms and Digital Imaging and Communication in Medicine (DICOM) images have been obtained for most of the 4439 patients treated with radiation therapy who were enrolled into the study [79].

Future Developments

It is important to understand how inherited genetic variants modify the risk for the development of normal tissue toxicities and subsequent malignancies following radiation therapy with photons or charged particles. Work in this area has tremendous potential to provide insight into radiation-related late effects, including carcinogenesis. This research is particularly important given estimates that the number of long-term cancer survivors will increase substantially in the coming years [80, 81]. In addition, knowledge that an individual may be at increased risk for developing radiation-induced adverse effects could alter the risk-benefit assessment of front-line therapy options. The information could also highlight those survivors who would most benefit from cancer prevention interventions or intensive surveillance. Before these clinical applications can be realized, however, it is essential that initial discoveries are replicated in independent populations with sufficient sample sizes. Additionally, potential modifiers of any gene and radiation therapy interaction must be investigated, including the type of radiation therapy (photon versus CPT). Results from large-scale ongoing studies, particularly those involving RGC collaborators for radiation therapy–induced toxicities and in children and young adults for subsequent malignancies, hold great promise for the future. Findings from these studies should provide important insights into the role of genetic susceptibility in photon and CPT-related development of these adverse effects.

There are enormous opportunities for radiogenomic studies of CPT given recent advances in technologies, the reduction in costs for genome-wide analyses, and experience gained over the past 9 years from the RGC and epidemiologists investigating radiation therapy–related second malignancies. A significant development for radiogenomic research is the creation of a biorepository containing clinical data and biospecimens collected by the PCG with anticipated growth resulting from participation of investigators associated with the Pediatric Proton Consortium Registry and Particle Therapy Cooperative Group. These important initiatives need to be expanded upon at an international level to build on the experience gained from RGC investigators with a goal of identifying the genomic factors associated with the development of adverse effects following cancer treatment with charged particles.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of Interest Statement: The authors have no conflicts of interest to disclose.

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