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Radiogenomics: Identification of Genomic Predictors for Radiation Toxicity

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Abstract

The overall goal of radiogenomics is the identification of genomic markers that are predictive for the development of adverse effects resulting from cancer treatment with radiation. The principal rationale for a focus on toxicity in radiogenomics is that for many patients treated with radiation, especially individuals diagnosed with early stage cancers, the survival rates are high and therefore a substantial number of people will live for a significant period of time beyond treatment. However, many of these patients could suffer from debilitating complications resulting from radiotherapy. Work in radiogenomics has greatly benefited from creation of the Radiogenomics Consortium (RGC), which includes investigators at multiple institutions located in a variety of countries. The common goal 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 markers. A principal aim of research in radiogenomics is the development of a predictive instrument to enable identification of people who are at greatest risk for adverse effects resulting from cancer treatment using radiation. It is anticipated that creation of a predictive assay characterized by a high level of sensitivity and specificity will improve precision radiotherapy and assist patients and their physicians to select the optimal treatment for each individual.

Introduction

The goals of research in radiogenomics fall into two general areas. The first main objective being pursued by investigators in this field is identification of genomic markers, primarily single nucleotide polymorphisms (SNPs) that could serve as the basis of an assay to predict the relative susceptibility for newly diagnosed cancer patients to develop adverse effects if they were to be treated with radiation [1, 2]. SNPs represent a major source of genetic variation between individuals as approximately once every 1,000 nucleotides more than 5% of people have an alternate base pair at a particular nucleotide [3], although the frequency of specific SNPs depends on ethnic, racial and geographic location. In addition, as the costs for whole

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exome and whole genome sequencing continue to decrease [4–6], it is likely that information will increasingly become available for many subjects in radiogenomic studies as to the presence of rare variants, which may be associated with various outcomes resulting from radiotherapy [7]. It has come to be recognized that patient-related characteristics, including genomic factors, could represent an important basis influencing susceptibility for development of radiation-related toxicities [8]. It should be noted that adverse effects resulting from radiotherapy are relatively common, with approximately 2–5% of patients developing some form of grade 3 complication and 10–20% experiencing moderate grade 2 toxicity [9]. Although great strides have been made to localize the dose of radiation to the cancer, normal tissues and organs still often are subjected to a substantial dose of radiation as part of treatment, which can result in significant complications. While radiotherapy is often curative, the adverse effects resulting from treatment can place a major financial burden on both individuals as well as the health care system [10].

Although the emphasis of research in radiogenomics has been on the identification of SNPs associated with outcomes, it is likely that epigenetic and other “panomic” factors [11] are also of importance and likely to be eventually incorporated into any predictive instrument that is developed as it evolves and improves in sensitivity and specificity. Nevertheless, the development of a SNP-based test would enhance precision radiotherapy as it will enable selection of patients who might benefit from a strictly surgical or drug treatment or use of a more conformal form of radiotherapy that spares normal tissues. Use of such a genetic/genomic predictive assay could enhance the therapeutic index through a decrease in the rate of complications. In addition, it may be feasible to dose escalate and possibly improve the cure rate for patients predicted to be at lower risk for radiation-induced injuries.

The second main aim of radiogenomics, which represents a more far-reaching goal, is the use of information gained through radiogenomic research to assist with the development of agents that could prevent or mitigate normal tissue/organ toxicities that may result from treatment with high doses of radiation. As genes are identified whose encoded products are affected by SNPs that reside either within or near these genes, it will then be possible to conduct mechanistic and functional studies to enhance an understanding as to the potential role that these gene products play in the development of adverse outcomes resulting from exposure to radiation. Thus, it is anticipated that a greater understanding of the molecular pathways that play a role in the development of radiation-injuries could lead to the development of pharmacologic agents with a capability to either prevent or mitigate these toxicities. However, progress towards this overall goal is dependent upon the validation of SNPs in multiple cohorts that have been discovered as associated with normal tissue toxicities resulting from cancer radiotherapy.

Factors that Facilitate Research and Challenges in Radiogenomics

Among the positive factors that facilitate a radiogenomics approach are that;

1. The outcome of interest occurs in response to a specific exposure, radiation - This is in contrast to many candidate gene and genome-wide association studies (GWAS) that are performed to identify genetic variants associated with an

increased probability to develop a certain disease for which there may be numerous environmental and life-style factors that could influence the probably an individual will develop a particular disease in addition to genetic influences.

2. Adverse effects resulting from cancer radiotherapy are relatively common - This factor enables studies of a more modest size to be performed compared with research focused on relatively rare phenotypes.

3. Findings of radiogenomic studies are actionable - For example, men diagnosed with prostate cancer now often have limited information in terms of basing a treatment decision following diagnosis with prostate cancer as to whether radiotherapy, surgery or active surveillance represents the best course of action. However, a man receiving the results of a predictive assay suggestive of radiosensitivity may opt for surgery or active surveillance if his PSA and Gleason scores are consistent with this recommendation. Conversely, men predicted to be at low risk for the development of toxicities following prostate radiotherapy may consider a more aggressive treatment. Similarly, a treatment decision for women diagnosed with early stage breast cancer may not be clear as to a choice between mastectomy or lumpectomy followed by radiotherapy. For women predicted to be at high risk for fibrotic responses in the breast and other adverse effects, mastectomy rather than radiotherapy may be advisable, especially since adverse radiation effects may compromise the ability for breast reconstruction [12]. However, women at low risk may feel greater confidence to proceed with limited surgery followed by radiotherapy. Young people diagnosed with Hodgkin's disease, and their families, are also often faced with a difficult decision as to whether radiation should be used to treat their cancer in addition to chemotherapy [13–15]. The cure rates for these forms of cancer, particularly when diagnosed at an early stage, are relatively high. Thus, many of these individuals will live for a substantial length of time following treatment. However, for those patients receiving radiotherapy, their risk for development of a serious complication or secondary malignancy caused by radiation exposure could be increased. Thus, development of a robust predictive assay would allow patients diagnosed with these and other forms of cancer to reach a more informed treatment decision.

4. A predictive assay can guide radiotherapy treatment planning - For patients predicted to be at a substantially greater risk for the development of adverse effects resulting from exposure to radiation, more stringent steps could be taken to limit the exposure of normal tissues and organs to radiation. One approach could be use of protons or carbon ions since patients at high risk might specifically benefit from the more conformal treatment that these alternate, but generally more costly forms of radiotherapy, may enable.

5. Biological insight can lead to development of preventative or mitigating agents - Although development of a predictive instrument to help guide treatment is the initial priority of research in radiogenomics, once genetic variants associated with radiation toxicities have been identified and validated in multiple cohorts, it

would be beneficial to embark upon mechanistic and functional studies to help elucidate the molecular basis as to the pathways through which an alteration in the products of these genes results in an increased susceptibility for dysfunctionality to particular organs or tissues. Such information could lead to the development of agents to help prevent or mitigate adverse outcomes following radiotherapy.

Challenges in Radiogenomics

Among the challenges of studies focused on radiogenomics are;

1. Dosimetry matters –It is important to obtain detailed treatment and dosimetric data for multivariable modeling. Unfortunately, this is not routinely accomplished for many studies and is a particular problem when attempting to combine data from multiple studies. This is a critical aspect of the REQUITE project [16] in which a series of dosimetric parameters, including the full DVHs (dose volume histograms) and DICOM (Digital Imaging and Communications in Medicine) images have been obtained for the roughly 5,000 patients treated with radiotherapy that have been enrolled into this study.
2. Need baseline (pre-radiotherapy) symptom assessment – In order to determine the impact of radiation in terms of symptoms that appear following treatment, it is essential to obtain baseline information for patients prior to the initiation of therapy. For example, men diagnosed with prostate cancer often already suffer from different forms of urinary or sexual function problems before being exposed to high doses of radiation as part of their treatment. It is therefore essential to know the change experienced by each subject from a base-line score.
3. Long-term follow-up (≥2yrs) - The development of symptoms varies with time and grade of effect. As opposed to studies whose aim is the identification of genetic variants associated with susceptibility for a particular disease, in which the outcome is essentially dichotomous, the development of adverse effects resulting from radiotherapy varies with both time and grade of complication. The question often arises as to whether a patient should be evaluated at a specific time point and/or grade level of toxicity or if the data should be analyzed as a continuous variable.
4. Variability in tools used to measure adverse effects – When combing multiple cohorts, as is often done in radiogenomic studies, different measures and scales to quantify toxicities are commonly employed across the centers contributing biospecimens and/or data to a particular project. It can therefore be difficult to harmonize outcomes. Although this heterogeneity has likely limited the discovery of many genetic variants, clearly it has not completely prevented their identification as there is often adequate similarity and overlap between the evaluation instruments used by different groups of investigators to enable successful association studies.

5. ‘Outcomes’ are multiple and incompletely understood – Multiple measures of toxicity for a particular outcome are routinely employed. For example, urinary toxicity in men treated for prostate cancer can be measured using hematuria, nocturia, straining, urgency and other end-points. In addition, many factors influence toxicity including dose, volume irradiated, time, co-morbid conditions and interaction with other modalities. Therefore, multi-variable analyses of radiogenomic studies are essential since multiple factors will influence the development of different forms of toxicity. One important effort to combine different forms of toxicity into one score has been attempted in what is termed a Standardized Total Average Toxicity (STAT) score [17].

6. Requirement for large sample sizes and multiple cohorts- Previous GWAS that have been performed demonstrate the need for large sample sizes [18–20]. This is of importance since the relative risk associated with any particular SNP is generally relatively small. In addition, in order to identify SNPs with a low minor allele frequency, large sample sizes are needed. Beyond that, multiple cohorts are essential in order to validate SNPs that were discovered through an initial GWAS. An important factor that has helped to address the need for multiple cohorts of large sample size was creation of the Radiogenomics Consortium (RGC), which was established in 2009 [21] and is a National Cancer Institute/ NIH-supported Cancer Epidemiology Consortium. The RGC currently consists of 217 investigators at 123 member institutions in 30 countries. The shared goals of the RGC members are to bring together collaborators for potential projects to share data and biospecimens so as to increase the statistical power of radiogenomic studies. The RGC has also facilitated the performance of cross-center validation studies, which are indispensable for the development of a predictive assay to gain widespread clinical acceptance [22].

Nevertheless, despite these many challenges, an increasing number of well-performed radiogenomic candidate gene studies and GWAS have been successfully performed, which are now producing an increasing number of genetic markers that have been discovered and validated in multiple cohorts as outlined below.

SNPs that have been Identified and Validated

The initial research performed in radiogenomics involved candidate gene studies, which focused on genes encoding proteins with known associations to pathways involved in responses to radiation, such as DNA repair processes and cell cycle checkpoint control. Although a number of positive associations were reported, these studies often did not adequately correct for multiple hypothesis testing and generally were not validated in subsequent studies [23], with several exceptions. The main advance in radiogenomics research has been achieved through use of SNP microarrays and the performance of GWAS in which large numbers of SNPs across the genome have been evaluated. Using this approach, several large GWAS and CGS have been accomplished involving a rigorous analysis for association between particular SNPs and specific outcomes. The results of these studies are summarized in Table 1.

Prostate Cancer

A series of studies examining common SNPs in candidate genes were initially performed but little evidence was obtained to validate any of the SNPs examined. However, once the cost substantially diminished for genotyping using DNA microarrays, the focus of research in radiogenomics shifted towards the performance of GWAS. It should be noted that due to the necessity to employ a correction for multiple-hypothesis testing, genome-wide significance for a GWAS is generally thought to be met only for those SNP associations with a p-value$<5\times10^{-8}$.

The first GWAS performed in radiogenomcs was to identify SNPs associated with erectile dysfunction in African-American men treated with radiotherapy for prostate cancer [24]. Through this study, a SNP (rs2268363) in the *FSHR* gene, which encodes follicle stimulating hormone, was identified ($p=5.46\times10^{-8}$). This hormone is expressed in Sertoli cells located in the testis and is involved in the development and function of this organ [25, 26]. Disturbance of the FSHR signaling pathway can result in small testis size, abnormal spermatogenesis and infertility.

A three-stage GWAS was conducted [27] using discovery and replication cohorts that included the use of STAT score [17] as the measure of combined adverse effects following prostate cancer radiotherapy. A locus encompassing the *TANC1* gene was associated with STAT score for overall late toxicity with an odds ratio (OR) of approximately six (combined $p=4.64\times10^{-11}$). The product encoded by the *TANC1* gene plays a central role for recruitment of fusion-competent myoblasts during myotube formation. It is biologically plausible that TANC1 is involved in the regeneration of muscle injured by radiation since it plays a necessary role to regenerate adult muscle in response to local damage [28].

Several forms of toxicity were examined in GWAS that focused on a cohort of approximately 800 patients treated with radiotherapy for prostate cancer [29–31]. Rectal bleeding as the outcome was the focus of one study in which two SNPs, rs7120482 and rs17630638, (p-values=5.4×10^{-8} and 6.9×10^{-7}, respectively) were identified. These SNPs lie upstream of the *SLC36A4* gene, whose product can impact the action of the mTOR complex 1 signaling pathway, which plays a role in radiosensitization, proliferation and cell survival [32–35]. Twelve SNPs were also discovered and validated that reside within or in close proximity to genes associated with the development of erectile dysfunction following radiation treatment (p-values 2.1×10^{-5}-6.2×10^{-4}). One of the strongest associations for erectile dysfunction was with SNP rs11648233 (OR= 1.8, $p=9.1\times10^{-5}$). This SNP is located within the *HSD17B2* gene whose product functions in oxidative metabolism of androgens and estrogens [36]. In addition, an 8-SNP haplotype block was identified exhibiting an association with change in American Urological Association Symptom Score (AUASS). The strongest association was for SNP rs17779457 (combined $p = 6.5\times10^{-7}$). This haplotype block is located within the *IFNK* gene whose product is a type I interferon that regulates cytokine release, which could influence the development of urinary complications following treatment of prostate cancer with radiation since these factors could play a role in the inflammatory response resulting from damage to tissues exposed to high doses of radiation [37]. Also, SNP rs13035033 that is located in *MYO3*B, which encodes actin-based

motor protein myosin IIIB and is highly expressed in the kidney, was associated with urinary straining ($p=5.0\times10^{-9}$)[38].

A replicated study involving men who received prostate cancer radiotherapy identified a SNP (rs2788612) located in the *KCND3* gene that was strongly associated with late rectal incontinence (RR=9.91, $p=1.05\times10^{-12}$) [39]. *KCDN3* encodes a member of the potassium channel, voltage-gated, shal-related subfamily, which is expressed in smooth muscle and thus may play a role in sphincter function [40].

A fixed-effect meta-analysis was performed using data from four cohorts consisting of 1564 men treated for prostate cancer for which a GWAS was performed in which toxicity was measured at a two-year time point [41]. Two SNPs were identified in this study that met genome wide significance. One was rs17599026, which resides on chromosome 5q31.2 and associated with urinary frequency and characterized by an OR of 3.1 (95% confidence interval 2.1–4.7, $p=4.2\times10^{-8}$). This SNP is located in an intronic region of *KDM3B*, 23bp downstream of exon 20. This gene is highly expressed in bladder tissue [42], which is consistent with a potential role for the encoded protein playing a role in normal bladder function. Thus, its alteration may increase the likelihood for a urinary complication upon exposure to a high dose of radiation.

rs7720298, which resides on chromosome 5p15.2, was associated with decreased urine stream with an OR of 2.7 (95% CI 1.9–3.9, p-value=3.2×10^{-8}). This SNP is located in an intronic region downstream of *DNAH5* exon 30. This gene encodes the dynein, axonemal, heavy chain 5 protein which is part a protein complex that is associated with microtubule formation. Mutations in *DNAH5* can result in primary ciliary dyskinesia resulting from abnormal cellular cilia and flagella [43].This gene is expressed in both the kidney and bladder, consistent with a possible role in the function of these organs [42]. Therefore, a variant in *DNAH5* may enhance the probability for an adverse urinary effect following radiotherapy. In addition to identification of a strong association between these two SNPs with urinary morbidity following radiotherapy, an important aspect of this study is the demonstration that meta-analysis of a multi-cohort consisting of subjects who were evaluated using variable toxicity instruments is able nevertheless to yield results identifying genetic markers of importance for outcomes following cancer treatment with radiation.

Breast Cancer

An increasing focus for radiogenomics investigators is the identification of SNPs associated with the development of adverse normal tissue outcomes resulting from radiotherapy of breast cancer. One such example was a study in which over 2,000 breast cancer patients from four cohorts treated with radiotherapy were genotyped for SNPs related to the TGFβ pathway and associations reported for several outcomes, including breast induration, telangiectasia and overall toxicity [44]. Significant and replicated associations with adverse outcomes following breast radiotherapy were reported for the *TNFα* SNP rs1800629 and rs2857595, which is located 25.7 kb from rs1800629 and resides in the intergenic region between *NCR3* and *AIF1*.

Another validated study of breast cancer patients identified SNP rs1139793 in *TXNRD2* associated with subcutaneous fibrosis following radiotherapy [45]. *TXNRD2* encodes the mitochondrial selenoprotein thioredoxin reductase 2, which plays a central role preventing oxidative damage [46]. Thus, alteration of the protein encoded by this gene could impact upon reactive oxygen species produced through irradiation and therefore influence the risk for fibrosis development following radiotherapy.

A separate study used a two-stage design to investigate associations between SNPs in genes whose products are involved with responses to oxidative stress with toxicities following radiation treatment of approximately 2,600 women diagnosed with breast cancer [47]. The rs2682585 SNP in *XRCC1* was found to be associated with risk for skin toxicities (OR 0.77, 95% CI 0.61–0.96, p=.02) and STAT score (−0.08, 95% CI −0.15 to −0.02, p=.016). The protein encoded by *XRCC1* plays a role in base excision repair of oxidative damage produced by radiation [48].

A GWAS was performed in which more than 1,500 patients who received radiotherapy for breast cancer were examined for SNPs associated with adverse effects resulting from treatment [39]. The quantile-quantile (Q-Q) plots from this study revealed a larger number of associations at the $p<5\times10^{-7}$ level than would be expected by chance. This result provides evidence that common genetic variants are associated with risk for development of adverse effects following radiotherapy.

A study of more than 5,000 patients treated for either breast or prostate cancer with radiotherapy reported an association between the rs1801516 in the *ATM* gene with ORs of 1.5 for acute and 1.2 for late toxicity [49].

Lung Cancer

It was reported in studies of patients treated with radiotherapy for non-small cell lung cancer (NSCLC) that the *HSPB1* rs2868371 SNP was associated with grade 3 or greater radiation pneumonitis [50] (p=0.02) and that this SNP was also associated with the development of grade 3 or greater radiation-induced esophagitis [51] in both training (p=0.045) and validation cohorts (p=0.031). HSP27 is a heat shock protein whose plasma concentrations are under genetic control of HSBP1 [52, 53]. HSP27 increases cellular resistance to heat shock, oxidative stress and inflammatory mediators. In addition, HSP27 additionally increases the antioxidant activity in cells and limits the toxicity of oxidized proteins through its chaperone activity. Thus, it is plausible that modulation of HSP27 levels through genetic alterations in *HSPB1* could impact the sensitivity of NSCLC patients for the development of lung pneumonitis and esophagitis following radiotherapy. In addition, it was reported that the *TGFβ1* rs1800469 SNP was associated with a higher risk of radiation esophagitis in both the training (p=0.045) and validation (0.023) sets of NSCLC patients [54]. Radiation can activate TGFβ1 from its latent form, which plays an important role in the etiology of radiation-induced inflammatory processes [55–58].

Model Development

An important factor in development of a radiogenomic predictive instrument is the creation of a suitable model. One approach is to build upon a normal tissue complication (NTCP) model, which has its basis dosimetric parameters, with the addition of genetic information and other patient-specific factors [59, 60]. Several predictive models have been created using the EMLasso technique [61], which represents a statistical approach for model building. This methodology helps to avoid over/under-fitting, includes cross-validation, employs the smallest number of parameters and is appropriately designed for datasets with missing values, a situation common in radiogenomic research. This technique has been used for several studies, including predictive models for dysphagia resulting from head and neck cancer treatment [62], genitourinary toxicities following treatment of prostate cancer with radiotherapy [63] and esophagitis following chemo-radiation treatment [64]. In addition, decision analytic methods such as decision curve analysis and net benefit can assist with the quantification of clinical usefulness [65, 66]. Several other approaches have also been suggested to evaluate radiation-induced normal tissue effects based on dosimetric and clinical factors, including logit-equivalent uniform dose and relative seriality [67], nearest-neighbor prediction [68] and the Lyman-Kutcher-Berman model [69].

Another key factor to consider for development of models is that normal tissue radiosensitivity for any particular tissue or organ is a complex trait dependent upon the expression of multiple genes whose variation is a reflection of the collective impact of numerous sequence variants [70, 71]. Hence, susceptibility for the appearance of adverse effects in a specific organ is likely the manifestation of several molecular pathways, which can be impacted by the presence of SNPs in multiple genes. Therefore, any predictive instrument to be developed will need to incorporate a multi-SNP component.

An important aspect associated with model building is that this type of analysis provides insight as to the number of SNPs that will be necessary to create a clinically useful predictive instrument. One approach to address this issue has been through the employment of simulation data [72], which is informative as to the robustness of predictive models for discrimination between individuals at high risk for development of complications following radiotherapy and those at low risk [73]. Several conclusions were obtained from these simulation experiments, including; (1) Inclusion of SNPs present in the genome with a high risk allele frequency and larger effect size enhances the accuracy of the model, (2) Increasing the number of SNPs included in a risk model improves the discrimination accuracy as quantitated through use of the area under the curve (AUC) for a receiver-operating characteristic curve and (3) High AUC values can be achieved through use of 50–100 common risk SNPs with effect sizes of 1.05–1.5. Substantial progress in radiogenomics research is being made towards discovery and validation of this number of SNPs. The results of these simulations are reassuring since they indicate that a relatively modest number of SNPs could form the basis of a clinically useful instrument capable of predicting risk for development of a particular form of toxicity resulting from cancer radiotherapy. It is therefore anticipated that a predictive test should be available for clinical utilization in the near future. Such an instrument should substantially improve upon and assist the clinical decision-making process.

Design of Clinical Trials

Now that substantial progress has been achieved in radiogenomics to identify biomarkers associated with development of adverse effects resulting from radiotherapy and advances have been realized towards model building, efforts are being focused on optimal design and patient selection for interventional trials using radiogenomic biomarkers [74]. One important point to consider in the design of clinical trials is that unlike disease susceptibility, the risk for radiation-induced toxicities is continuous for development of toxicities with an increasing incidence of complications with larger radiation doses and/or volumes irradiated. In addition, a SNP-predictive assay will likely require one or multiple thresholds for classifying risk into discrete categories. Thus, a classical biomarker trial design may not be appropriate, while an approach using a risk factor stratification methodology could be more suitable.

Current Research and Future Directions

Three large studies are currently in progress whose main goal is to discover new SNPs and validate previously identified genetic biomarkers predictive of susceptibility for the development of adverse effects resulting from radiotherapy. One such study involves roughly 6,000 men treated for prostate cancer, which encompasses multiple cohorts created by RGC investigators. DNA samples from all of these men have been genotyped using a GWAS chip and detailed clinical data are available with a minimum of two-years of follow-up. The goals of this project are to; (1) Discover new SNP associations and validate previously identified SNPs linked with the development of adverse outcomes resulting from radiotherapy, (2) Build clinically useful multi-SNP models that incorporate dosimetric and clinical factors to predict susceptibility for the development of toxicities following radiotherapy and, (3) Develop a low-cost, high performance assay and companion risk assessment tool to predict risk for development of complications resulting from treatment with radiation. Related to this aim, research is being conducted that is supported by the NIH Small Business Innovation program [75] to help rapidly translate the findings from this project into an assay ready for implementation in the clinic and routine medical care.

A second large project is REQUITE (Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality-of-life in cancer survivors)[16]. This is a multi-center study involving member investigators of the RGC. An important aspect of this project is that it addresses the problem of data harmonization, which is a significant challenge in most radiogenomic studies that involve multiple cohorts in which the subjects were followed using a variety of evaluative instruments. For REQUITE, identical categories of clinical and dosimetric information were obtained for all subjects and the same health professional and patient reported outcome forms were used at all enrolling centers. The objectives of REQUITE are to; (1) Perform a multi-center, observational cohort study in which epidemiologic, treatment, longitudinal toxicity and quality of life data are collected from approximately 5,000 patients treated with radiotherapy for either breast, prostate or lung cancer, (2) Produce a centralized biobank in which DNA is isolated from patients enrolled in the observational study and create a centralized data management system for secure collection, integration, mining, sharing and archiving of all project data, (3)

Validate published SNP biomarkers of radiosensitivity and discover new variants associated with specific forms of adverse effects following radiotherapy, (4) Validate clinical/dosimetric predictors of radiotherapy toxicity and incorporate biomarker data, (5) Design interventional trials to reduce long-term adverse cancer treatment effects and, (6) Deliver interventional trial protocols using validated models incorporating biomarkers to identify patient sub-populations likely to benefit from interventions and to serve as a resource exploitable for future studies exploring relationships between adverse effects resulting from radiotherapy and the genetics of radiosensitivity using developing technologies such as next generation sequencing.

A third project involves roughly 4,500 women treated for breast cancer with radiotherapy in which blood samples and detailed clinical and follow-up information are available. These come from three cohorts in which blood samples, treatment, dosimetric and follow-up data have been obtained for approximately; (1) 1,000 women treated as part of RTOG 1005, which is a trial examining the use of a hypofractionated protocol with a concurrent boost for treatment of breast cancer [76] , (2) 2,000 women enrolled into the REQUITE study [16] and, (3) 1,500 subjects from a series of clinical protocols performed at New York University School of Medicine [77–80].

It is anticipated that the results of these three large projects in radiogenomics will result in the discovery of new SNPs and the validation of previously identified genetic markers, which will form the basis of an assay to predict outcomes from radiotherapy that will be ready for application in routine cancer care.

Conclusion

Substantial progress in radiogenomic research has been achieved towards the creation of a test predictive of the susceptibility for individual cancer patients as to the development of adverse effects resulting from radiotherapy, which often have a deleterious impact upon the quality of life for these individuals. It is also likely that identification of SNPs and genes whose encoded products play a role in the molecular etiology of the development of radiation-induced toxicities will advance our understanding as to the molecular basis through which these adverse outcomes arise. It is expected that this knowledge should assist in the development of agents to prevent or mitigate these radiation-induced injuries. Thus, it is anticipated that clinical implementation of a predictive instrument characterized by a high level of sensitivity and specificity will substantially enhance the ability to select an optimal treatment for people diagnosed with cancer and thereby improve outcomes and advance precision radiotherapy.

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Table 1

SNPs Associated with Adverse Effects following either Prostate, Breast or Lung Cancer Radiotherapy

SNP Number	Gene	Gene Product	Function	Adverse Effect	GWAS or CGS	p-value	Odds Ratio (95% CI)	Reference
PROSTATE								
rs2268363	*FSHR*	follicle stimulating hormone	Expressed in Sertoli cells located in the testis; involved in the development and function of the testis	erectile dysfunction	GWAS	5.4×10^{-8}	7.0 (3.4–14.7)	24
rs264663	*TANC1*	tetratricopeptide repeat, ankyrin repeat and coiled-coil domain-containing protein 1	Recruitment of fusion-competent myoblasts during myotube formation	overall late GU and GI toxicity	GWAS	4.6×10^{-11}	6.6 (2.2–19.6)	27
rs7120482	upstream of *SLC36A4*	solute carrier family 36 member	High-affinity/low-capacity non-proton-coupled amino acid transporter; modulate s action of the mTOR complex 1 signaling pathway	rectal bleeding	GWAS	5.4×10^{-8}	3.1 (1.7–5.6)	30
rs17630638						6.9×10^{-7}	2.9 (1.6–5.2)	
rs11648233	*HSD17B2*	hydroxysteroid 17-β-dehydrogenase 2	Oxidative metabolism of androgens and estrogens	erectile dysfunction	GWAS	9.1×10^{-5}	1.8 (1.2–2.8)	29
rs17779457	*IFNK*	type I interferon	Regulates cytokine release	AUA symptom score	GWAS	6.5×10^{-7}	2.4 (1.1–3.6)(BC)	31
rs13035033	*MYO3B*	myosin IIIB	Actin-based motor protein; highly expressed in the kidney	urinary straining	GWAS	5.0×10^{-9}	0.9 (0.6–1.2)(BC)	
rs2788612	*KCND3*	potassium voltage-gated channel, shal-related subfamily member 3	Regulates epithelial electrolyte transport, smooth muscle contraction and cell volume	late rectal incontinence	GWAS	1.1×10^{-12}	9.9 (RR)	39
rs17599026	*KDM3B*	lysine demethylase 3B	Histone demethylase; specifically demethylates Lys-9 of histone H3	urinary frequency	GWAS	4.2×10^{-8}	3.1 (2.1–4.7)	41
rs7720298,	*DNAH5*	dynein, axonemal, heavy chain 5	Part of a protein complex associated with microtubule formation	decreased urine stream		3.2×10^{-8}	2.7 (1.9–3.9)	
BREAST								
rs1800629	*TNFα*	tumor necrosis factor alpha	Multifunctional proinflammatory cytokine	telangiectasia	CGS	0.0028	NP	44
rs2857595	Intergenic region between *NCR, 3* and *AIF1*	NCR3-natural cytotoxicity triggering receptor 3; AIF1-allograft inflammatory factor 1	NCR3-natural cytotoxicity receptor that may aid NK cells in the lysis of tumor cells. AIF1-induced by cytokines and interferon; may promote macrophage activation and growth of vascular smooth muscle cells and T-lymphocytes	overall late toxicity	CGS	0.01	2.0 (1.0–3.9)	
rs1139793	*TXNRD2*	thioredoxin reductase 2	Selenocysteine-containing flavoenzyme; maintains thioredoxins in a reduced state, thereby playing a key role in regulating the cellular redox environment.	subcutaneous fibrosis	CGS	0.012	3.2 (1.3–8.2)	45
rs2682585	*XRCC1*	X-ray repair cross complementing 1	Base excision repair of oxidative damage	skin toxicities	CGS	0.02 (CGS)	0.8 (0.6–1.0)	47

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SNP Number	Gene	Gene Product	Function	Adverse Effect	GWAS or CGS	p-value	Odds Ratio (95% CI)	Reference
rs1801516	*ATM*	ATM telangiectasia mutated	Serine/threonine protein kinase that is recruited and activated by DNA double-strand breaks; phosphorylates several key proteins that initiate activation of the DNA damage checkpoint, leading to cell cycle arrest, DNA repair or apoptosis	overall toxicity	CGS	NP	1.2 (1.0–1.4)	49
				overall acute toxicity			1.5 (1.2–1.9)	
				acute skin toxicity			1.7 (1.1–2.7)	
				telangiectasia			1.3 (1.1–1.7)	
				fibrosis			1.3 (1.0–1.6)	
LUNG								
rs2868371	*HSPB1*	heat shock protein family B (small) member 1	Involved in stress resistance and actin organization; translocates from the cytoplasm to the nucleus upon stress induction	Pneumonitis	CGS	0.02	NP	50
				esophagitis	CGS	0.045 (TC)	0.29 (0.09–0.97)	51
						0.031 (VC)	(TC;HR) 0.25 (0.07–0.88) (VC;HR)	
rs1800469	*TGFβ 1*	transforming growth factor beta 1	Multifunctional protein that controls proliferation, differentiation and other functions in many cell types.	esophagitis	CGS	0.045 (TC)	2.5 (1.0–6.0)	54
						0.023 (VC)	(TC;HR) 2.5 (1.1–5.6) (VC;HR)	

AUA, American Urological Association; BC, beta coefficient; CGS, candidate gene study; CI, confidence interval; GI, gastrointestinal; GU, genitourinary; GWAS, genome wide association study; NP, not provided; RR, relative risk; HR, hazard ratio; TC, training cohort; VC, validation cohort