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REVIEW ARTICLE

Radiation biology and oncology in the genomic era

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ABSTRACT

Radiobiology research is building the foundation for applying genomics in precision radiation oncology. Advances in high-throughput approaches will underpin increased understanding of radiosensitivity and the development of future predictive assays for clinical application. There is an established contribution of genetics as a risk factor for radiotherapy side effects. An individual's radiosensitivity is an inherited polygenic trait with an architecture that includes rare mutations in a few genes that confer large effects and common variants in many genes with small effects. Current thinking is that some will be tissue specific, and future tests will be tailored to the normal tissues at risk. The relationship between normal and tumor cell radiosensitivity is poorly understood. Data are emerging suggesting interplay between germline genetic variation and epigenetic modification with growing evidence that changes in DNA methylation regulate the radiosensitivity of cancer cells and histone acetyltransferase inhibitors have radiosensitizing effects. Changes in histone methylation can also impair DNA damage response signaling and alter radiosensitivity. An important effort to advance radiobiology in the genomic era was establishment of the Radiogenomics Consortium to enable the creation of the large radiotherapy cohorts required to exploit advances in genomics. To address challenges in harmonizing data from multiple cohorts, the consortium established the REQUITE project to collect standardized data and genotyping for ~5,000 patients. The collection of detailed dosimetric data is important to produce validated multivariable models. Continued efforts will identify new genes that impact on radiosensitivity to generate new knowledge on toxicity pathogenesis and tests to incorporate into the clinical decision-making process.

INTRODUCTION

Radiotherapy is a cornerstone of modern cancer treatment and is used in curative and palliative care for over half of cancer patients.¹ As with any cancer treatment, the goal of radiotherapy is to maximize tumor control while minimizing damaging effects on surrounding normal tissues. The extent to which this goal is achieved is represented by the therapeutic ratio; *i.e.* the ratio of tumor control probability to normal tissue complication probability. Improvement in the therapeutic ratio can be achieved by, *e.g.*: (1) improved targeting of radiation to the tumor using imaging technology and conformal dose delivery (2) designing radiation protocols that exploit differences in biology between tumor cells and normal tissues; and (3) use of radioprotective and/or radiosensitizing agents. These approaches have been employed over the past decades, following progress

in our understanding of radiation biology and in radiation delivery technology. For example, advances in three-dimensional conformal radiotherapy and intensity modulated radiotherapy in the 1990s enabled more precise targeting of the tumor and sparing of normal tissue.² Image-guided radiation therapy now uses multidimensional imaging during the course of radiotherapy to adapt the treatment plan to changes in patient position and tumor and normal tissue changes during the course of treatment.³ Knowledge of radiobiologic characteristics of tumor and normal tissues has led to development of alternative fractionation protocols, such as hypofractionation,^{4–7} which takes advantage of differences in the α/β ratio between tumor and normal tissues. Oligofractionation and stereotactic ablation radiotherapy/stereotactic body radiotherapy deliver ever-higher radiation doses in fewer fractions with the intention of

improving tumor cell killing, but which may affect toxicity risk differently from standard fractionation.⁸ In addition, with the increasing use of charged particle therapy, primarily in the forms of protons and carbon ions, there is a need to establish cohorts for patients treated with these advanced technology forms of radiotherapy and to create biorepositories with linked clinical data. While some genetic variants are likely to impact toxicity 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. Increased understanding of radiobiologic mechanisms has also begun to result in development of promising radiosensitizing and radioprotective agents.⁹

Radiation oncology is poised to enter the era of individualized cancer care. A novel approach that could be used to improve the therapeutic ratio in radiation oncology is to tailor treatment to an individual's tumor and/or normal tissue response to radiation. A predictive assay to identify those at greatest risk for normal tissue response could, for example, identify those patients who could most benefit from proton therapy, thus maximizing the cost-benefit ratio of this treatment. A predictive assay might also be used to identify those patients who are at low risk for normal tissue toxicity and could safely be treated with hypofractionation or high dose stereotacticablation radiotherapy/stereotactic body radiotherapy. Another use would be to select patients in whom radioprotectors or radiosensitizers might be most necessary and/or beneficial.

It has long been recognized that substantial variation exists among patients in the incidence and severity of normal tissue reactions to radiotherapy. Similarly, some tumors of a particular type, grade, and stage are eliminated by a given dose of radiation whereas others recur. Evidence from *in vitro*, animal, and human studies suggests that this variation is primarily due to differences in genetic background among individuals and the cancers these individuals develop, as well as differences in epigenetic alterations and downstream effects on RNA and protein expression, cellular behavior, microenvironment and systemic response to radiation. Advances in knowledge and technology in the fields of genomics, transcriptomics, proteomics, metabolomics and other high throughput approaches has led to increasing interest in understanding the genetic and biologic basis of radiosensitivity and developing predictive assays for use in the clinic. This review focuses on recent advances in the study of genomics and epigenomics for normal tissue and tumor response to radiation. We touch briefly on the role of inflammatory biomarkers, and the reader is referred to recent reviews for other related biomarkers and functional assays of radiosensitivity.^{10–13} It will also describe areas of potential clinical impact. Recent findings and ongoing efforts will be highlighted.

GENETIC BIOMARKERS OF RADIOSENSITIVITY

Heritability of radiosensitivity

Radiation oncology has a long history of research and clinical interest in understanding the genetic basis for individual variation in response to treatment and personalizing therapy. A better understanding of the genetic basis would uncover novel biologic

pathways important in radiation response. In addition, specific genetic variants could serve as biomarkers indicative of the expression of normal-tissue toxicity. Such biomarkers could be used in the predictive setting, as markers that are measurable prior to radiation exposure and whose level could be used to predict how normal tissues might respond to radiotherapy. Individual variation of normal tissue response for a given radiation dose was first described formally in 1936,¹⁴ with the publication of the sigmoid dose response curve. This curve showed a near-Gaussian frequency distribution of individual sensitivity for the development of skin telangiectasia.¹⁵ The possibility of using a cellular or gene-based assay has been discussed for decades,^{16,17} as has been the potential impact in clinical outcomes.^{18–20} The hypothesis that the basis for this variation may be genetic, at least in part, is supported by the existence of several rare genetic syndromes associated with hypersensitivity to radiation. For example, mutations in *ATM* result in ataxia-telangiectasia, a syndrome characterized by extreme radiosensitivity and increased risk for developing cancer.²¹ 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.^{22,23}

However, the variable responses seen in patients not affected by one of these rare syndromes, who are treated with protocols involving similar dosimetric characteristics, suggests the importance of *common* genetic factors. *In vitro* studies of apoptotic response or chromosome damage following irradiation have estimated the heritability of cellular radiosensitivity to range from 58 to 82%,^{24–29} and these estimates are in agreement with clinical estimates of normal tissue effects following radiotherapy.³⁰ Like other human traits, normal tissue response to therapeutic radiation is considered to be a complex polygenic trait, in which many common single nucleotide polymorphisms (SNPs) and rare variants have a combined effect on modulating the response of cells and tissues to radiation exposure.

Genetic association studies

Initial studies aimed at identifying SNPs associated with normal tissue reactions focused on candidate genes identified from radiobiologic studies of cellular and tissue response to DNA damage, free radical metabolism, wound healing, and inflammation. Studies sufficiently large in sample size that have included both discovery and replication sets have been successful in identifying validated SNP-toxicity association. For example, a study of SNPs that lie in genes involved in reactive oxygen species metabolism found that the missense SNP rs1139793 in *TXNRD2* was significantly associated with radiation-induced fibrosis in breast cancer survivors.³¹ The genotype of rs1139793 was significantly associated with mRNA expression level of *TXNRD2* in blood, suggesting a functional impact of the SNP. In a candidate gene study of radioresponse pathways including DNA damage repair, inflammation and oxidative stress response, rs1800629 near the inflammatory cytokine *TNF* displayed a replicated association with breast toxicity (induration, telangiectasia, edema and atrophy).³² In another example, a study of non-small cell lung cancer patients reported that the functional promoter

variant rs2868371 in *HSPB1* was significantly associated with radiation pneumonitis.³³ This variant was also associated with radiotherapy-induced esophagitis, indicating it may play a broad role in radiosensitivity across multiple tissue types.³⁴ A recent meta-analysis of rs1801516 in *ATM* showed an increased risk of overall radiotherapy-induced acute and late toxicity,³⁵ again suggesting that some SNPs may play a broad role in radiosensitivity across multiple tissue types. In contrast, although a replicated association between rs1800469 in *TGFB1* and esophagitis was reported,^{36,37} a large meta-analysis of individual patient data ($N = 2,782$, 11 independent studies) reported no association between rs1800469 and radiotherapy-induced fibrosis,³⁸ suggesting that there may be tissue specificity, at least for this particular SNP.

With more recent advances in genomic technology, genome-wide association studies (GWAS) have begun to uncover additional, novel radiation toxicity loci within genes not considered in candidate SNP studies. The first pilot radiogenomics GWAS identified SNPs within *FSHR* that were associated with erectile dysfunction following radiotherapy for prostate cancer,³⁹ providing proof of principle that this method could be used to identify risk loci. Subsequently, a larger three-stage GWAS of late toxicity following radiotherapy for prostate cancer identified a locus in *TANC1* associated with overall genitourinary and gastrointestinal toxicity.⁴⁰ A recent meta-analysis of four GWAS identified two more SNPs associated with increased risk of developing urinary toxicity following radiotherapy for prostate cancer within *KDM3B* and *DNAH5*.⁴¹ Although premature to speculate on the functional consequences of these SNPs, they lie within genes that are expressed in the bladder, where radiotherapy can lead to increased urinary frequency and decreased urine stream in males treated for prostate cancer. Though a GWAS requires large sample sizes to achieve adequate statistical power for SNP discovery, it does not require *a priori* assumptions about the genes or pathways involved in the outcome. Thus, GWAS can be a powerful tool for uncovering novel radiobiologic mechanisms.

Cellular radiation response involves many of the same biologic mechanisms and pathways involved in carcinogenesis, including DNA damage repair, metabolism of reactive oxygen species, inflammation, and cell migration. This observation has led investigators to hypothesize that germline genetic variants known to be associated with increased risk for developing cancer may also be associated with increased normal tissue toxicity following cancer treatment with radiotherapy. This would be of concern in the clinical setting, as cancer patients being treated with radiation are more likely to harbor germline cancer risk variants than the general population. There are several hundred known cancer risk variants that have been identified through large consortium-led GWAS, particularly through the iCOGs^{42–44} and Oncoarray⁴⁵ initiatives. Two recent studies investigated this hypothesis and found that neither a polygenic score for prostate cancer⁴⁶ nor a polygenic score for breast cancer⁴⁷ showed a significant association with increased risk of developing late radiotherapy toxicity in these respective populations. It is important to note that these analyses considered only common germline SNPs—those with a minor allele frequency $\geq 1\%$ and therefore, do not

rule out a role of rare cancer risk variants in radiation toxicity. Indeed, several such rare variants are known which lead to the syndromes of increased cancer risk and severe radiosensitivity, as described above. Similarly, genomic copy number alterations are commonly observed in tumors in genes that affect double strand break repair pathways, *e.g.* *BRCA1* *BRCA2*, *ATM*, *CHEK2* and those associated with Fanconi anemia.⁴⁸ Heterozygous mutations in these genes may be present in the germline and affect normal tissue repair of DNA double strand breaks. Tumors in mutations carriers might also have enhanced radiosensitivity, given the central role of the double strand break repair pathway in radiation response. Further studies are needed to investigate the effect of these types of alterations on radiation response in both tumor and normal tissue.

Radiation response is a somewhat unique example of gene–environment interaction. Normal tissue toxicities and tumor cell killing occurs specifically in response to an environmental exposure: ionizing radiation. Thus, the effect of a particular genetic variant may vary depending on the radiation dose. Indeed, evidence of this effect has begun to emerge. A study of rare mutations in *ATM* found evidence of gene–environment interaction in development of contralateral breast cancer following exposure to radiation.⁴⁹ Carriers of deleterious mutations in *ATM* who were exposed to therapeutic radiation for treatment of initial breast cancer are at increased risk of developing contralateral breast cancer compared with carriers of deleterious mutations in *ATM* who did not receive radiation for treatment of their initial breast cancer, and the risk ratio increases with increasing radiation dose.⁴⁹ In the GWAS of prostate cancer patients treated with pelvic radiotherapy described above,⁴⁰ a statistical interaction was detected for SNPs in the *TANC1* locus and total radiation dose.⁴⁰ Another GWAS identified genetic variants near *PRDM1* that were associated with increased risk of developing a second malignancy in survivors of pediatric Hodgkin's lymphoma who were treated with radiotherapy as children, and this effect was not seen in those treated with radiotherapy as adults, suggesting interaction between *PRDM1* and exposure to ionizing radiation that could also depend on developmental stage.⁵⁰

Genetic architecture of radiosensitivity

The results from genetic association studies are shedding light on the genetic architecture of radiosensitivity, *i.e.* the types of alterations, distribution of allelic effects and patterns of pleiotropy, dominance, and epistasis. For example, truncating and other non-synonymous mutations in *ATM* produce a non-functional protein that impairs response to DNA damage induced by radiation and consequently development of severe normal tissue toxicity.⁵¹ Similarly, non-synonymous mutations in *NBS1* and *LIG4* directly affect protein function and cause the radiosensitivity syndromes associated with these genes.^{22,23} Genetic variants that greatly impact the function of essential proteins tend to be rare. Less than 1 to 3% of the population is estimated to be carriers of deleterious variants of *ATM*, with far fewer homozygous individuals who display severe radiosensitivity.⁵² It is plausible that additional single-gene syndromes remain to be identified, but given that severe toxicity is rare, and genetic variants with large effect sizes will be rare, different study designs will

be required to uncover such associations. Advances in next-generation genomic sequencing now make it feasible to identify rare variants through modestly sized case-control studies of patients selected from the tails of the radiosensitivity distribution: those with severe radiation toxicities and those who are radio-resistant. In contrast, *common* SNPs that affect coding regions of genes tend to have more subtle effects on protein function and consequently on risk for disease or phenotype, enabling their detection in large cohort studies including patients with mild/moderate radiosensitivity. For example, SNP rs1801516 in *ATM*, mentioned above,³⁵ which results in a non-conservative amino acid substitution from an aspartic acid to an asparagine in exon 37, has a minor allele frequency of 16% in Europeans and increases risk for developing radiation-induced toxicity only moderately (odds ratio of approximately 1.5 for acute toxicity and 1.2 for late toxicity). Because of the subtle effect of this common SNP, the association was only detected when assessed in a sample of over 5000 radiotherapy patients.

It should be noted that the majority of disease-associated common SNPs are located in regulatory regions such as promoters, enhancers, regions of chromatin conformation control, and splice sites,⁵³ and a similar genetic architecture is hypothesized to underlie normal tissue radiosensitivity. Variants in these regions generally have more subtle effects on biologic pathways than protein-coding variants. For example, gene enhancer elements, characterized by open chromatin flanked by sites of histone methylation are involved in regulating transcriptional programs,⁵⁴ and SNPs within these regions can determine whether they are active (associated with the acylation of lysine 27 of histone 3 [H3K27Ac]) or repressed (correlated with histone marks H3K27Me3 and H3K9Me3). Thus, a risk SNP may subtly affect gene expression without directly altering the coding sequence of the gene. Often when a disease-associated locus is first identified using genome-wide methods, the functional impact of the locus is not immediately clear. The locus tagged by rs17599026, identified by the GWAS meta-analysis described above,⁴¹ encompasses a region that includes the promoter of *KDM3B* and could potentially affect gene expression levels. Fine-mapping and subsequent functional studies are underway to elucidate the biologic effect of this locus.

EPIGENETICS AND RADIOSENSITIVITY

While early efforts in radiogenomics focused primarily on germline genetic variants, the role of chromatin modification in radiation response has garnered increased interest in recent years and is of great potential clinical importance. As mentioned above, germline genetic variants could serve as predictive biomarkers of normal tissue toxicity, measured prior to exposure. Epigenetic marks could also serve as biomarkers in this context. In addition, due to the dynamic nature of epigenetic changes, epigenetic marks might also apply in the setting of early detection of ongoing damage at the cellular level that occurs during or after radiation exposure that might eventually manifest as normal tissue toxicity. In this setting, such biomarkers could be used to screen for normal tissue damage that might be treated early with mitigating agents or minimized by dosimetric changes during the course of radiotherapy. In addition, while studies of genetic

variants have mostly investigated associations with normal tissue outcomes, epigenomic research also focuses on tumor response to radiation. These two areas could thus be complementary in informing our understanding of an individual's tumor and normal tissue radiosensitivity profile. There are, in addition, some examples beginning to emerge that suggest interplay between germline genetic variants and epigenetic modification, as mentioned above in the context of the effect of common SNPs on gene regulatory regions.

Many chromatin modifications have been shown to affect DNA damage response (DDR) signaling and repair, which is a critical mechanism underlying cellular radiosensitivity in both tumor and normal tissues. Among the types of chromatin modifications, histone methylation and acetylation and DNA methylation are the most widely characterized and hence, have been the major focus of novel therapeutic strategies.

DNA methylation

Growing evidence suggests that changes in DNA methylation may be involved in regulating the sensitivity or resistance of cancer cells to radiation. Genomic hypomethylation by inhibition of one or more DNA methyltransferases has been observed to alter cell radiosensitivity. The DNA methyltransferase inhibitor 5-azacytidine (5-Aza) enhances radiation sensitivity in colon cancer cells^{55,56} and can sensitize radioresistant laryngeal cancer cells to irradiation, suggesting that changes in DNA methylation contributed to their radioresistance.⁵⁷

It has been reported that differential DNA methylation in gene promoters or enhancers affects the risk of late normal tissue radiotoxicity. For example, DNA hypermethylation of the promoter region of *RAD51* family genes *XRCC2* and *RAD51L3* increased the risk of late toxicity in cervical cancer patients after chemoradiotherapy.⁵⁸ Also, hypomethylation in the enhancer region of *DGKA*, encoding diacylglycerol kinase alpha, was associated with radiation-induced fibrosis in cancer patients.⁵⁹

In addition, there are several reports of altered DNA methylation levels following exposure to ionizing radiation in human cell lines and mice.^{60–64} Together with the supporting evidence for altered promoter methylation in some cancers,⁶⁵ these data provide a connection between radiation exposure, epigenetics, and both cancer and normal tissue outcomes.

Histone acetylation

Several histone acetyltransferase (HAT) inhibitors such as curcumin and anacardic acid have been reported to radiosensitize cancer cells, suggesting HATs play a role in controlling cell radiosensitivity. Lack of the TIP60 HAT impairs DNA repair and sensitizes cancer cells to radiation, implying that TIP60 inhibitors could be combined with radiotherapy to improve cancer control, though the role of this pathway in normal tissue radiosensitivity is not known.⁶⁶ For example, anacardic acid was found to inhibit TIP60-dependent activation of ATM and DNA-PK induced by DNA damage and sensitize tumor cells to ionizing radiation.⁶⁷ Inhibition of various histone deacetylases (HDAC) has also been shown to sensitize a variety of cell lines and xenografts to the

effects of radiation. The HDAC inhibitor vorinostat behaves as a radiosensitizer in many cancer cell lines, such as non-small-cell lung cancer, prostate, melanoma, osteosarcoma and glioma.^{68–73} The molecular mechanism of HDAC inhibition in cellular radiosensitization involves the upregulation of p21 in a p53-independent manner, leading to cell cycle arrest in the G1 phase.⁷⁴ The mechanism through which HDAC inhibitors butyrate and trichostatin A function may be to suppress cyclins A and D, stabilize p21 mRNA, and trigger p16 and p27, initiating cell arrest.^{75–77} In response to irradiation following HDAC inhibition, suppression of *ATM* gene expression and signaling has also been shown.^{78,79} HDAC inhibition can also induce the expression of pro-apoptotic genes that encode for proteins such as Apaf-1, Bmf, Bim, TRAIL, DR5, Bax, and TP2 and downregulate antiapoptotic genes resulting in a decrease in proteins including BCL2, MCL1 and XIAP.⁸⁰ It is plausible that this mechanism could affect cellular radiosensitivity given that radiation-induced DNA damage can trigger an apoptotic response. Importantly, the effect of HDAC inhibition on apoptotic pathway genes seems to be specific to tumor cell lines, suggesting an attractive approach to targeting cancer while sparing normal tissues. HDAC inhibition has also been reported to cause prolonged radiation-induced γ H2AX foci in cells following DNA repair inhibition.^{81,82}

HDAC inhibitors are promising as radiosensitizers, since they target tumor cells but have minimal effects on normal cells.^{79,83} A recent report showed that the HDAC inhibitor panobinostat radiosensitized bladder cancer xenografts *in vivo* but did not increase radiation-induced intestinal and bladder toxicity, partly because panobinostat preferentially accumulates in xenografts relative to plasma.⁸⁴ Panobinostat is an efficient radiosensitizer in the nanomolar range *in vitro* associated with downregulation of the DNA damage signaling proteins MRE11 and NBS1 and the HR protein RAD51.⁸⁵ The radiosensitizing effects of panobinostat might be primarily mediated via its Class I HDAC-selective effects, in particular, through its effects on HDAC1 and 2, which are highly expressed in cancer cells.⁸⁴ Although panobinostat has undergone Phase I clinical trial evaluation as a radiosensitizer, more Class I-selective HDAC inhibitors should be developed preclinically, as they may have less systemic side effects, and such studies might lead to clinical trials.

Histone methylation

Histone methylation marks and methyltransferases are emerging as important players in DDR following irradiation. Reduction in the levels of histone methylation on specific residues could impair DDR signaling and result in radiosensitization.⁸⁶ For example, loss of H3K9me3 by inhibiting both Suv39h1 and Suv39h2 methyltransferases suppressed ATM activation and led to cell radiosensitization.⁸⁷ In addition, the MLL methyltransferase is associated with chromosomal translocations resulting in the generation of chimeric fusion proteins and unusual recruitment of DOT1L, which plays an important role in 53BP1 recruitment and also may affect NHEJ-mediated repair.⁸⁸ Targeting DOT1L might, therefore, also provide a mechanism through which the therapeutic effect of radiotherapy could be enhanced. Studies of the lysine-specific histone demethylase *KDM1* and some Jumonji C family members in DDR signaling suggest that targeting

histone demethylase activity could also impact DDR, resulting in radiosensitization.^{89–92} This mechanism is of particular interest given that the previously described GWAS meta-analysis of late radiotherapy toxicity in prostate cancer patients identified SNP rs17599026, located in *KDM3B*, as significantly associated with the development of late urinary toxicity.⁴¹ Unpublished data from our group (laboratory of Dr K-H C) demonstrate that *KDM3B* knockdown can selectively protect non-neoplastic prostate and bladder urothelial cells from radiation-induced cell death without protecting prostate cancer cells from lethal effects of radiation. Transcriptome and quantitative RT-PCR analyses further demonstrates that *KDM3B* knockdown significantly induces multiple DSB-repair genes and strongly suppresses multiple pro-inflammatory genes in normal prostate and bladder urothelial cells but not in prostate cancer cells. Therefore, targeting *KDM3B* has potential to be an effective molecular therapy aimed at ameliorating normal tissue toxicity in prostate cancer patients receiving radiotherapy without impacting tumor control. These findings represent an early example of how results from genetic association studies in clinical patient populations can be translated back to the bench to uncover novel radio-response mechanisms.

ROLE OF CYTOKINES AND THE INFLAMMATORY MICROENVIRONMENT IN TUMOR AND NORMAL TISSUE RADIOSENSITIVITY

As described above, one area of emphasis in early candidate genetic association studies was SNPs within genes encoding cytokines and related proteins involved in inflammation. The reason for this focus is that normal tissue response to radiotherapy is similar in many ways to wound healing,⁹³ involving an inflammatory signaling environment that varies across individuals and could explain, in part, the patient-to-patient variation in normal tissue reactions. There is also evidence that the inflammatory microenvironment can affect tumor radiosensitivity. Thus, cytokines, and by extension genetic and epigenetic variants determining cytokine gene expression, represent an attractive family of biomarkers that could be used in prediction of an individual's normal tissue and/or tumor response to radiation. Indeed, as cited above, replicated associations with late radiotherapy toxicity have been reported for SNPs tagging the pro-inflammatory cytokine *TNF*³² and the proliferative cytokine *TGFB* genes.^{36,37}

The first studies of serum inflammatory cytokines in radiotherapy response were conducted by Rubin et al in a mouse model of lung irradiation.⁹⁴ They found that levels of the inflammatory cytokine IL1 α and the proliferative cytokine TGFB fluctuated over the course of radiation exposure and correlated with development of normal tissue damage. Subsequent studies in clinical patient populations provided additional evidence that levels of serum cytokines vary across patients and may be associated with radiotherapy response. For example, an analysis of a panel of circulating cytokines collected from patients receiving thoracic radiotherapy identified IL-1 α and IL-6 as early circulating cytokine markers for radiation pneumonitis.⁹⁵ Similarly, C-reactive protein (CRP) is a well-studied, acute-phase reactant, which serves as a sensitive and specific marker of tissue

damage and inflammation,⁹⁶ and emerging evidence suggest CRP may be a marker of treatment response. Circulating CRP levels have been associated with many solid tumor prognoses, particularly in genitourinary malignancies.⁹⁷ Elevated CRP recently has been associated with lower overall survival in metastatic castrate-resistant prostate cancer and a lower-response probability to chemotherapy.^{98,99} In the context of radiotherapy, it has recently been demonstrated that higher CRP levels correlate with worse cancer outcomes in radiotherapy-treated patients with non-metastatic prostate cancer.¹⁰⁰ These retrospective data provide hypothesis-generating support for the fact that the host inflammatory state at diagnosis may influence a prostate cancer patient's response to treatment with radiation therapy. There are also several additional inflammatory cytokines that are related to the CRP pathway that have been examined in patients with adenocarcinoma of the prostate. An early study of cytokine levels in prostate cancer showed that increasing IL-2 and IL-1 expression were associated with increased probability of acute gastrointestinal and genitourinary toxicity, respectively.¹⁰¹ Examples of other inflammatory cytokines that have been investigated in prostate cancer include GM-CSF,¹⁰² IFN-Gamma,¹⁰³ IL-1 beta,¹⁰⁴ IL-2,¹⁰⁵ IL-4,¹⁰⁶ IL-8,¹⁰⁷ IL-10,¹⁰⁸ IL-12,¹⁰⁹ MCP-1,^{110,111} and TNF-alpha.¹¹¹ Additional research is needed to understand the precise interaction between systemic levels of such inflammatory cytokines and the host response to radiation therapy. It will also be important to characterize the inter patient variation in inflammatory response and to determine the genetic and epigenetic basis for such variation.

PROGRESS TOWARDS CLINICAL UTILITY & FUTURE DIRECTIONS

An important effort to advance the field of radiogenomics was establishment of the Radiogenomics Consortium (RGC) whose purpose is to enable the creation of large patient cohorts that received radiotherapy. The RGC is a National Cancer Institute/NIH-supported Cancer Epidemiology Consortium (<https://epi.grants.cancer.gov/radiogenomics/>) through the Epidemiology and Genomics Research Program^{112,113} and consists of 225 investigators at 131 institutions in 31 countries. The goal of the RGC is to develop a collaborative infrastructure to permit large-scale discovery GWAS and validation studies that are essential for the identification of genetic factors associated with responses to radiotherapy. The RGC helps link investigators with common interests to pursue collaborative studies with large samples sizes so as to increase the statistical power of this research. It is also a goal of the RGC to conduct integrated clinical studies in which genetic variants along with multiple additional factors, including epigenetic makers, mRNA expression and serum inflammatory markers are investigated simultaneously and create models predictive of radiotherapy outcomes. Although the RGC has successfully assembled large cohorts to perform adequately-powered studies,¹¹⁴ data harmonization remains a challenge for studies involving multiple cohorts consisting of patients treated with a variety of radiotherapy techniques and evaluated using multiple grading systems. In addition, blood samples from most existing studies were collected at varying times during routine follow up after treatment, precluding the ability to examine epigenetic marks, mRNA, and cytokines whose levels

change over time, vary across tissues and cell types, and require standardized collection methods.

In order to address these deficiencies, the REQUITE (Validating predictive models and biomarkers of radiotherapy toxicity to reduce side-effects and improve quality-of-life in cancer survivors) project was launched.¹¹⁵ REQUITE is a large multicenter study involving member investigators of the RGC. A critical aspect of this project is that it addresses the problem of data harmonization as it mandates that identical clinical and dosimetric data are obtained for all subjects and standardized health professional and patient reported outcome forms used at all enrolling centers. A key aspect of REQUITE was translating and validating CTCAE-based patient reported outcome questionnaires into multiple languages, which have been used alongside widely-use EORTC quality-of-life questionnaires. REQUITE enrolled 4442 patients treated with radiotherapy for either breast, prostate or lung cancer and has produced a centralized biobank and database. Efforts are underway to validate published SNP biomarkers and clinical/dosimetric predictors of radiosensitivity and discover new variants associated with radiotherapy outcomes. REQUITE is also developing interventional trial protocols using validated models incorporating biomarkers to identify patient subpopulations likely to benefit from interventions and to serve as a resource exploitable for future studies. It is of particular importance that detailed treatment and dosimetric data for multivariable modeling are being obtained through REQUITE, as this information is unfortunately not routinely collected for many studies and is a particular problem when attempting to combine data from multiple studies.

In addition to the comprehensive toxicity data collected, cancer type-specific information is obtained for all participants enrolled into the REQUITE study. These data include patient characteristics (e.g. age, sex, height, bra size, smoking history, alcohol consumption), co-morbidities (e.g. diabetes, collagen vascular disease, history of heart disease, medication use), tumor factors (e.g. stage, histology), other treatments (use of surgery, chemotherapy and other systemic treatment) and radiotherapy (e.g. technique, fractionation, dose volume histogram parameters). The detailed patient history is obtained pre-treatment and updated post-treatment on an annual basis for a minimum of 2 years following completion of radiotherapy (this information is also obtained at 3 and 6 months for lung cancer patients in recognition that their relatively poor prognosis may result in an inability to obtain data at longer time points). Tumor response information is also collected post-treatment. The specific dosimetric parameters obtained for breast cancer patients were maximum skin dose, mean heart dose, hot spots (>107% of prescription dose), internal mammary volume and ipsilateral lung dose. For males who received radiotherapy for prostate cancer, the dosimetric information included rectal V30-V80, bladder V50-V78, large bowel V50, femoral head V50 and penile bulb V50 and V60. If the patient also received brachytherapy, then details as to whether it was either HDR or LDR, dose, source, rectal D1cc and urethral V125 and V150 were acquired. The information obtained for lung cancer patients were the V5, V20 and mean dose for the lung; the mean dose, D1cc and

the V35, V50 and V60 for the esophagus; and the mean dose, D1cc and the V5, V30, V40 and V50 for the heart. In addition to this comprehensive dosimetric information obtained for all subjects enrolled into REQUITE, the full dose volume histograms and DICOM images are stored in the centralized database. At the time of writing this review, full genome genotyping was completed using the OncoArray-500K BeadChip and the pre-treatment blood samples collected. These SNP genotyping data will be available on the database by September 2018. The centralized REQUITE resource also has breast photos before and 2 years following radiotherapy for the breast cancer patients.

SUMMARY

The research outlined in this paper highlights recent advances and progress in the development of genomic signatures and “-omics”-based tests for the prediction of tumor and normal tissue response to radiation treatment. Future efforts aimed at validating current signatures and incorporating additional information about tumor cell signaling, metabolism, the immune response, and imaging characteristics will further refine the predictive power, sensitivity, and ultimately the clinical utility of these tests. Additionally, continued efforts by the RGC to identify the genes and elucidate the functional impact of SNPs associated with normal tissue toxicity will facilitate the personalization

of radiation treatment delivery. Furthermore, the development of centralized databases and data collection standardization similar to that achieved for the REQUITE study will substantially enhance the identification of biomarkers predictive of outcomes resulting from cancer radiotherapy. Thus, it is anticipated that continued progress over the coming years will result in the incorporation of these molecular factors into the clinical decision-making process and thereby, improve the ability to personalize treatment decisions for cancer patients and enhance precision radiotherapy.

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