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Predicting Toxicity from Radiation Therapy- It's Genetic, Right?

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Just a few years after Wilhelm Röentgen discovered x-rays in 1895 and Antoine Becquerel discovered radioactivity in 1898, various forms of radiation were used to treat cancer. It was immediately apparent that radiation therapy held great promise as an effective therapeutic modality. It was also observed that radiation therapy damaged normal tissues, sometimes severely. Over the course of many years of investigation it was determined that the primary determinants of normal tissue toxicity are:

- **a.** Physical characteristics of the radiation beam (e.g. type of radiation, energy, dose rate)
- **b.** Time, dose, and fractionation (e.g. dose/treatment, total dose, fractionation schedule)
- **c.** Normal tissue irradiated (e.g. volume and type of normal tissue receiving a specified dose)
- **d.** Radiosensitizers (e.g. chemotherapy)
- **e.** Comorbidities (e.g. connective tissue disorders, prior surgery)

These factors provide clinicians a basic framework to assess the risk of toxicity in an individual patient and largely guide routine clinical decisions. However, even when all of the factors listed above are relatively constant, a wide range of responses to radiation therapy are observed. It is clear that inherited mutations in certain genes can lead to radiosensitivity. Individuals with rare genetic disorders such as ataxia telangiectasia and Nijmegen breakage syndrome develop severe reactions if exposed to therapeutic doses of radiation[1]. However, even for the vast majority of otherwise healthy patients receiving radiation therapy, genetic factors appear to play a significant role determining radiation response.

It is generally assumed that radiation sensitivity is a quantitative complex trait. Alterations in certain key genes (e.g. protein truncation mutations in both copies of *ATM*) can result in

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extreme radiosensitivity. However, it is expected that for the great majority of people, normal tissue toxicity is influenced by the cumulative effect of multiple genetic polymorphisms. Furthermore, it is likely that a degree of tissue specificity exists, whereby certain genes influence radiation sensitivity in one organ but not others. If these assumptions are true, the prediction of radiation-induced toxicity based on underlying genetics will be challenging.

In order to identify the genetic factors associated with the development of radiation-induced toxicity, with a goal to create an assay to predict radiotherapy response, candidate gene studies have been conducted. With this approach, SNPs in a series of genes associated primarily with DNA repair pathways and other radiation-related processes were genotyped in subjects that exhibited radiation-associated toxicities. The results of this work have been summarized in several excellent recent reviews[2–5]. An example of a candidate gene SNP approach was used by Mak et al. in this issue of CANCER. Their endpoint was clinical symptomatic radiation pneumonitis, typically consisting of shortness of breath and/or cough developing 1–6 months post-radiation therapy. They noted an association between the risk of pneumonitis and a SNP in the methylenetetrahydrofolate reductase gene (the AC or CC genotype having an increased risk compared to the AA genotype), which can be added to a list of results from similar studies (Table 1). The growing number of studies in this area perhaps reflects our collective frustration in being unable to accurately predict pneumonitis risk based on clinical and dosimetric parameters alone. While well-intentioned, these studies illustrate some of the fundamental shortcomings in this area of research. A brief review of these studies specifically evaluating lung toxicity provides some insight into the difficulties faced in understanding and assessing the inherited risk of radiation sensitivity.

Suboptimal Endpoint Selection and Scoring

The endpoint in most of these studies was clinical radiation pneumonitis, which was scored predominantly in a retrospective fashion. This is problematic, since it is notoriously difficult to accurately identify and grade treatment-related toxicity based upon the medical record. Furthermore, radiation pneumonitis is essentially a diagnosis of exclusion- there is no test that can be done to confirm the diagnosis. Even prospectively, it is often difficult to distinguish it from other common entities such as exacerbation of chronic obstructive pulmonary disease, pneumonia, and cardiac disease[6]. Thus, the endpoint used in most of these studies is somewhat subjective. A similar problem plagues the literature addressing the role of SNPs in other radiation-associated toxicities that also largely rely on somewhat subjective, non-specific symptom-based endpoints.

Confounding Variables

The risk of radiation pneumonitis is clearly related to the volume of lung exposed to various doses of radiation[7], and may also be affected by a variety of clinical factors (e.g. chemotherapy, pre-treatment lung function). The impact of these factors on the pneumonitis risk is challenging to quantify. The typical multivariable analysis, while commonly used, might not adequately address these sources of variation. Thus, genetic-based studies should ideally be conducted on populations of relatively-uniform patients. However, this need is in

direct conflict with the requirement to include a large number of patients. For example, it is well known that frequencies of SNPs vary significantly between ancestries, which may influence susceptibility to diseases and complications from treatments. While some of the studies confined their investigation to a single self-reported ancestry, most did not. When multiple ancestries, with different underlying polymorphism frequencies are combined, this complicates interpretation of the data set. Of course, to maintain as much power as possible, it is tempting to include all eligible patients, regardless of ancestry. It is also unclear if polymorphisms leading to toxicity in one ethnic or racial group will translate to a similar risk in a different group.

Validation studies

Most of the published studies lack validation in an independent replication cohort. This should be an essential requirement for publication of any study purporting to demonstrate an association between a particular SNP with a radiotherapy outcome.

Power

Perhaps the biggest criticism of the referenced studies is the statistical approach. The number of subjects in these studies is relatively small by the standards typically applied to such genetic-based research. Unless large numbers of patients are analyzed, the sheer size of the genome and the large number of SNPs would be expected to lead to a high false positive rate using a candidate gene approach. The primary obstacle to performing a genome wide association (GWA) study is sufficient patient numbers. Indeed, studies in which thousands of patients are genotyped may be necessary to have the statistical power to identify the polymorphisms which are independently contributing to radiation sensitivity. To provide a resource for the conduct of such large scale studies, which can only be accomplished through multi-institutional collaborations, an international Radiogenomics Consortium has been established[8, 9]. The goal of this consortium is to provide a structure in which tissue samples and data can be pooled from investigators performing work in radiogenomics on a global scale to create a large biorepository and databank, which will substantially strengthen the statistical power of these studies. Once initial studies have been performed and evidence for the involvement of specific genes has been obtained, the Radiogenomics Consortium will also play an important role for the essential validation studies in which additional radiotherapy cohorts will be genotyped.

Correction for Multiple Hypothesis Testing

Most studies involve genotyping multiple SNPs. However, a correction for multiple hypothesis testing is often not included with the authors simply taking note of this limitation and referring to their results as “hypothesis generating”.

Despite the shortcomings of these studies, their conduct reflects our need and desire to develop better predictors for radiation-induced normal tissue injury. One cannot always wait for “ideal study conditions” because they may never exist. For example, there is no expectation in the foreseeable future that we will be able to accurately quantify the impact of

various clinical factors on the risk for pneumonitis. Therefore, we conduct these types of research studies while acknowledging their limitations.

To address some of these limitations, our group at Duke/UNC has used imaging as an objective quantitative metric to assess radiation injury. Patients have undergone pre- and post-radiation CT and single photon emission computed tomography perfusion scans[10]. By registering the scans to each other and the 3D radiation dose distribution, we can define the degree of dose-dependent changes in the lung. This metric is likely independent of the specific dose/volume parameters (a major source of inter-patient variation that can confound analyses). Thus, we believe that this might provide an objective measure of inherent radiation sensitivity. However, as opposed to symptomatic radiation pneumonitis, a clinically relevant endpoint for the patient, asymptomatic imaging changes may be of little clinical consequence. Further, these imaging-based assessments are expensive, time consuming, and are thus not practical to perform in large numbers of patients. An ideal endpoint to assess radiation sensitivity would be objective, measurable, clinically relevant, and scored in a prospective fashion. Such endpoints are rare. A more scientifically sound approach is needed to address this issue.

Many candidate-gene studies have demonstrated a statistically significant association between various SNPs and radiation-induced normal tissue toxicity. However, none of the positive associations reported has been consistently validated in subsequent studies with other patient cohorts. Thus, at this time, no single gene or SNP has been identified that can serve as the basis for a predictive assay. The lack of success and limited scope of candidate gene studies has prompted GWA studies that more broadly search for genes and SNPs associated with radiation response. A fundamental reason for GWA studies is the recognition that only rudimentary knowledge exists as to the molecular pathways involved in the development of most normal tissue responses to radiation. It is therefore expected that a GWA approach will yield a useful clinical assay to predict radiation response, and that the affected genes may provide information to help elucidate the molecular pathways leading to radiation injuries. This knowledge may guide the development of pharmacologic agents to prevent or mitigate radiation-induced toxicity.

Recently, the first radiogenomics GWA study designed to identify SNPs associated with radiation-induced toxicity was published[11]. The study considered SNPs associated with erectile dysfunction among African American patients with prostate cancer treated with radiotherapy. The SNP most significantly associated with erectile dysfunction was located in the follicle stimulating hormone receptor gene (rs2268363), whose encoded product plays a role in male gonad development and function. This SNP is located in a gene associated with normal erectile function rather than one more specifically linked to radiation response. Since most candidate SNP studies address genes associated with radiation response, this gene would *not* have been identified using a candidate gene approach. While there is substantial enthusiasm and promise for GWA studies, the superiority of this over a candidate gene approach has not been demonstrated. Further work is underway.

While it is generally assumed that DNA sequence alterations play an important role in underlying susceptibility to radiation-induced toxicity, changes in gene *function* are also

likely contributing. For example, one study that compared intra- with inter-patient variation in skin telangiectasias concluded that approximately 80–90% of the variability in radiation response was due to patient-related factors[12]. In contrast, it was estimated that only 10–20% of the difference could be explained through stochastic (random) events arising from the probabilistic nature of radiation-induced cell killing and variations in dosimetry and dose delivery. While genetic makeup was assumed to be the primary patient-related factor accounting for inter-patient differences, epigenetic and other such phenomenon are probably also playing a role. For example, Kozin et al utilized an in-bred strain of mice and performed uniform irradiation to the legs to study tibial shortening[13]. Similar to the study on telangiectasias in humans, they found that leg shortening in mice was significantly influenced by variability between individuals. Because the mice in this experiment were genetically identical, this implies that individual factors other than genetic make-up are influencing radiation toxicity. If true, unraveling the contribution of epigenetic and genetic factors will pose an additional challenge.

In conclusion, the ultimate goal of these investigations is the personalization of a patient's treatment plan to maximize their therapeutic ratio. A patient's individual plan can be modified based on inherent differences in the sensitivity of their normal tissues. Patients deemed at a very high risk of radiation-related complications may be best treated with a surgical alternative if one were to exist, or smaller radiation fields, lower doses, or aggressive supportive care if radiation therapy remained the best local modality. It is possible that tumors in patients susceptible to radiation toxicity may also prove to be radiosensitive and cured with lower-than-standard treatment doses. On the other hand, patients at low risk of toxicity may benefit from radiation dose escalation to maximize the chance of local tumor control. While progress is being made, we remain far from the point where these data can be used in the clinical setting.

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References

1. Pollard JM, Gatti RA. Clinical radiation sensitivity with DNA repair disorders: an overview. Int J Radiat Oncol Biol Phys. 2009; 74(5):1323–31. [PubMed: 19616740]
2. Alsner J, Andreassen CN, Overgaard J. Genetic markers for prediction of normal tissue toxicity after radiotherapy. Semin Radiat Oncol. 2008; 18(2):126–35. [PubMed: 18314067]
3. Andreassen CN, Alsner J. Genetic variants and normal tissue toxicity after radiotherapy: a systematic review. Radiother Oncol. 2009; 92(3):299–309. [PubMed: 19683821]
4. Barnett GC, West CM, Dunning AM, et al. Normal tissue reactions to radiotherapy: towards tailoring treatment dose by genotype. Nat Rev Cancer. 2009; 9(2):134–42. [PubMed: 19148183]
5. Rosenstein BS. Identification of SNPs associated with susceptibility for development of adverse reactions to radiotherapy. Pharmacogenomics. 2011; 12(2):267–75. [PubMed: 21332318]
6. Kocak Z, Evans ES, Zhou SM, et al. Challenges in defining radiation pneumonitis in patients with lung cancer. Int J Radiat Oncol Biol Phys. 2005; 62(3):635–8. [PubMed: 15936538]

7. Marks LB, Bentzen SM, Deasy JO, et al. Radiation dose-volume effects in the lung. Int J Radiat Oncol Biol Phys. 2010; 76(3 Suppl):S70–6. [PubMed: 20171521]
8. West C, Rosenstein BS, Alsner J, et al. Establishment of a Radiogenomics Consortium. Int J Radiat Oncol Biol Phys. 2010; 76(5):1295–6. [PubMed: 20338472]
9. West C, Rosenstein BS. Establishment of a radiogenomics consortium. Radiother Oncol. 2010; 94(1):117–8. [PubMed: 20074824]
10. Kelsey CR, Jackson L, Langdon S, et al. A Polymorphism Within the Promoter of the TGFbeta1 Gene Is Associated With Radiation Sensitivity Using an Objective Radiologic Endpoint. Int J Radiat Oncol Biol Phys. 2011 epub May 21, 2011.
11. Kerns SL, Ostrer H, Stock R, et al. Genome-wide association study to identify single nucleotide polymorphisms (SNPs) associated with the development of erectile dysfunction in African-American men after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys. 2010; 78(5): 1292–300. [PubMed: 20932654]
12. Safwat A, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. Int J Radiat Oncol Biol Phys. 2002; 52(1):198–204. [PubMed: 11777639]
13. Kozin SV, Niemierko A, Huang P, Silva J, Doppke KP, Suit HD. Inter- and intramouse heterogeneity of radiation response for a growing paired organ. Radiat Res. 2008; 170(2):264–7. [PubMed: 18666813]
14. Yuan X, Liao Z, Liu Z, et al. Single nucleotide polymorphism at rs1982073:T869C of the TGFbeta 1 gene is associated with the risk of radiation pneumonitis in patients with non-small-cell lung cancer treated with definitive radiotherapy. J Clin Oncol. 2009; 27(20):3370–8. [PubMed: 19380441]
15. Zhang L, Yang M, Bi N, et al. ATM polymorphisms are associated with risk of radiation- induced pneumonitis. Int J Radiat Oncol Biol Phys. 2010; 77(5):1360–8. [PubMed: 20171797]
16. Yang M, Zhang L, Bi N, et al. Association of P53 and ATM polymorphisms with risk of radiation-induced pneumonitis in lung cancer patients treated with radiotherapy. Int J Radiat Oncol Biol Phys. 2011; 79(5):1402–7. [PubMed: 20729006]
17. Yin M, Liao Z, Huang YJ, et al. Polymorphisms of homologous recombination genes and clinical outcomes of non-small cell lung cancer patients treated with definitive radiotherapy. PLoS One. 2011; 6(5):e20055. [PubMed: 21647442]
18. Yin M, Liao Z, Liu Z, et al. Functional Polymorphisms of Base Excision Repair Genes XRCC1 and APEX1 Predict Risk of Radiation Pneumonitis in Patients with Non-Small Cell Lung Cancer Treated with Definitive Radiation Therapy. Int J Radiat Oncol Biol Phys. 2011
19. Yin M, Liao Z, Liu Z, et al. Genetic variants of the nonhomologous end joining gene LIG4 and severe radiation pneumonitis in nonsmall cell lung cancer patients treated with definitive radiotherapy. Cancer. 2011
20. Hildebrandt MA, Komaki R, Liao Z, et al. Genetic variants in inflammation-related genes are associated with radiation-induced toxicity following treatment for non-small cell lung cancer. PLoS One. 2010; 5(8):e12402. [PubMed: 20811626]

Table 1

Studies Evaluating Single Nucleotide Polymorphisms and Radiation-Induced Lung Toxicity

Study	N	Endpoint	Method of Assessment	Population	Approach	Genes Associated with Toxicity
Yuan[14]	164	Symptomatic Radiation Pneumonitis	Retrospective	77% White	Candidate gene	*TGFB1*
Zhang[15] Yang[16]	253	Symptomatic Radiation Pneumonitis	Prospective	100% Han Chinese	Candidate gene	*ATM* and *P53*
Yin[17–19]	165–228	Symptomatic Radiation Pneumonitis	Retrospective	71–73% White	Candidate Gene	*RAD51, XRCC1, APEX1, LIG4*
Hildebrandt[20]	173	Symptomatic Radiation Pneumonitis	Retrospective	100% non- Hispanic Caucasians	Candidate Gene	*IL1A, IL8, TNF, TNFRSF1B, MIF, NOS3*
Kelsey[10]	39	Radiological Changes	Prospective	100% White	Candidate gene	*TGFB1*
Mak	136	Symptomatic Radiation Pneumonitis	Retrospective	100% White	Candidate Gene	*MTHFR*