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Biomarkers and surrogate endpoints for normal-tissue effects of radiation therapy: the importance of dose-volume effects

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Abstract

Biomarkers are of interest for predicting or monitoring normal tissue toxicity of radiation therapy. Advances in molecular radiobiology provide novel leads in the search for normal tissue biomarkers with sufficient sensitivity and specificity to become clinically useful. This paper reviews examples of studies of biomarkers as predictive markers, as response markers or as surrogate endpoints for radiation side-effects. Single nucleotide polymorphisms (SNPs) are briefly discussed in the context of candidate gene and genome wide association studies. The importance of adjusting for radiation dose distribution in normal tissue biomarker studies is underlined. Finally, research priorities in this field are identified and discussed.

Keywords

biomarkers; normal tissue effects; predictive factors; toxicity; dose distribution; single nucleotide polymorphisms; surrogate endpoints; radiogenomics

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Conflict of Interest Statement

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1. Patient-to-patient variability in normal tissue response to radiation

Any group of patients will exhibit a range of normal tissue effects in response to an identical course of radiation therapy. It has long been debated whether this is mainly the result of a deterministic variation in radioresponsiveness[1] or a random (stochastic) variation in the induction and processing of damage[2]. Unique insights into this question have come from studies of the inter- and intra-patient variability in early and late side-effects in two separately irradiated fields in a large population of patients[3–5]. For example, one study estimated that as much as 81% of the total variation in the development of skin telangiectasia was attributable to deterministic effects[3]. It has also been shown that the within-patient correlations between the occurrence of two different late endpoints, fibrosis and telangiectasia, or between early and late endpoints appear to be low[4, 5]. These findings provide strong support for the hypotheses that: (1) there are biological determinants of the risk of normal tissue toxicity that varies among individuals, and (2) these factors are likely to be specific for a given radiation pathogenesis. It is noteworthy, in the QUANTEC (QUantitative Analysis of Normal Tissue Effects in the Clinic) context, that the studies cited above have analyzed skin and subcutaneous endpoints where a relatively well-defined reference dose can be assigned to the tissue of interest in a specific patient[6] due to the simple field techniques used. In contrast, modern radiation therapy techniques typically give rise to a broad range of absorbed doses in the tissues and organs of interest.

While much of the theoretical discussion has focused on the hypothetical existence of a distinct sub-population of individuals with a marked increase in radioresponsiveness, clinical data seem not to support this hypothesis[7]. A sub-group of patients expressing a given radiation side-effect at a relatively low dose would give rise to a “bump” at the foot of the sigmoid clinical dose-incidence curve for normal tissue reactions, an effect that has not been evident in a relatively large clinical series. This appears to imply that this hypothetical hyper-responsive clinical phenotype must either have a very low prevalence or they cannot be far separated from the majority of cases in terms of responsiveness.

Taken together, the above studies are consistent with a hypothesis promulgating considerable inter-patient variability in radioresponsiveness for a specific endpoint but reflective of a relatively broad continuum of varying responsiveness rather than a distinct sub-population of sensitive individuals. Stratification of patients according to the risk of toxicity could potentially guide modality selection or interventions to mitigate this risk in high-risk individuals or allow intensification of therapy in low-risk individuals. Also, biological variability in radioresponsiveness is likely to be a major confounding factor in dose-response and dose-volume analyses, associated with a loss of statistical resolution and potentially causing problems with model identification.

2. Proposed terminology: Biomarkers and surrogate endpoints

A *biomarker* is a measurable characteristic of a biological system that is indicative of normal function or disease state of the system or its response to an external factor such as a therapeutic intervention. While the literature on biomarkers in cancer biology and tumor therapy outcome is rich and rapidly expanding[8], the study of biomarkers in normal-tissue radiobiology remains a research field in its infancy. The development of new high-throughput assays as well as advances in molecular radiation pathology[9] is likely to boost research on toxicity biomarkers in the next five to ten years[10].

There is currently no general consensus on the appropriate terminology for biomarkers relevant to normal-tissue radiation research, but we propose distinguishing between three main classes of biomarkers: *predictive factors*, *response markers* and *surrogate endpoints*.

Predictive factors are biological or clinical factors (but not treatment-related factors) observable at baseline, i.e. before the start of therapy that are statistically associated with the probability of a given outcome of a specific treatment in an individual. In the case of normal tissue side-effects these are often called risk factors or protective factors. This is consistent with the definition in Okunieff et al.[11]. The distinction between predictive and prognostic markers is of major importance in tumor biology[8]. The analogous distinction for normal tissue risk factors would be between markers associated with poor tolerance to any (effective) therapy vs. markers predicting excess risk of toxicity after a specific therapy. While some factors could potentially be in the former group, performance status for example, most factors studied so far are modality specific and are therefore likely to be predictive rather than prognostic. Note that Okunieff et al. [11] essentially defines a prognostic marker as a predictive marker assessed after the start of therapy. However, this definition seems to be at variance with the traditional use of this term in cancer research.

Response markers are defined here as therapy-related changes in biomarkers that are mechanistically related to treatment outcome at the individual patient level. These are sometimes referred to as *direct markers* of the underlying pathologic process[12]. In the case of normal tissue toxicity these markers would ideally reflect a deterministic step in the radiation pathogenesis of a specific side-effect. Response markers may be used for guiding therapy intensity or interventions for toxicity in an individual patient.

Endpoints are health state characteristics that are used to assess treatment outcome in a population of patients. Clinical endpoints are symptoms, signs or functional measures of disease or toxicity. Clinical tumor endpoints are desirable treatment outcomes, reflecting the therapeutic aim, such as local tumor control or progression-free survival. Clinical normal-tissue endpoints are side effects affecting the patient's health-related quality of life. *Surrogate endpoints* are measurable biological effects that can be used as an early indicator of the effect of therapy on a given clinical endpoint in a population of patients. A response marker may serve as a surrogate endpoint. However, a surrogate endpoint might not necessarily be mechanistically related to the occurrence of the clinical endpoint in an individual (see below).

It is noteworthy that markers that are associated with a disease state may not necessarily be valid surrogate endpoints. One example is the Cardiac Arrhythmia Suppression Trial where clinical development of encainide and flecainide, despite demonstrating efficacy in suppressing arrhythmia after myocardial infarctions, were discontinued because of excess mortality compared with patients receiving a placebo[13]. Another example is low hemoglobin concentration in cancer patients, a biomarker shown to be associated with increased disease burden and poor therapeutic outcome. Administration of erythropoietin led to the desired increase in hemoglobin concentration, but proved in randomized placebo-controlled trials to be associated with a worse outcome[14].

3. Predictive markers

Predictive markers, assessed at baseline, i.e. before the start of therapy, are aimed at selecting cases for a specific type of therapy or for changing radiotherapy dose-fractionation prescription or planned dose distributions. In vitro radiosensitivity of normal human skin fibroblasts looked promising as a clinical radiosensitivity assay in the early 1980's. However, when large confirmatory studies were conducted a significant association was not found[15].

3.1 Cytokines and growth factors as predictive factors

The possible predictive value of cytokines and growth factors, involved in damage response and tissue remodeling assessed at baseline, have also been investigated in a number of studies. One example is transforming growth factor β-1 (TGF-β1), a strongly profibrotic, multifunctional cytokine[9] that can be activated from its latent form by ionizing radiation. This activation has been demonstrated within an hour after doses as low as 0.1 Gy. Li et al.[16] showed a statistically significant association between the level of TGF-β1 in pre-radiotherapy plasma samples and subsequent development of radiation fibrosis in 91 early-stage breast cancer patients, thus demonstrating that this is a potential predictive marker for this side-effect..

Baseline plasma levels of a number of pro-inflammatory cytokines have been studied as predictive markers for radiation side-effects by the group at University of Rochester. In one study, these investigators found that, interleukin (IL)-1α and IL-6, were elevated in 13 patients who went on to develop symptomatic radiation pneumonitis compared with 11 patients who did not[17]. The predictive power of IL-6 was slightly higher than that of IL-1α, however, the positive (PPV) and negative (NPV) predictive values for IL-6 were only 80% and 47%, respectively. The relatively disappointing results with such phenotypic assays have motivated studies into genotypic assays as an alternative in the post-Human Genome Project era.

3.2 Genetic variations

Advances in molecular biology, particularly with the advent of high-throughput assays, have stirred interest in radiogenomics, the study of the possible link between genotypic variation and radiation therapy toxicity. It has long been known that some rare genetic syndromes, such as Nijmegen breakage syndrome and ataxia telangiectasia (AT), are associated with hyper-radiosensitivity in vitro as well as in the clinic[9]. In particular, the ATM gene, mutated in patients with AT, has been intensively studied as a candidate gene of interest in radiogenomics. While individuals who are homozygous for ATM mutations, which generally cause truncation of the encoded protein, show dramatic radiation hyper-sensitivity, the very low incidence of AT (<1 per 40,000 live births) means that this syndrome cannot account for the normal tissue toxicities observed in a general population of patients receiving radiotherapy. The relative contribution of patients who are heterozygous for ATM protein truncation mutations to the spectrum of radiation reactions seen in an unselected population of patients remains unclear.

Recently, radiogenomic interest has focused on single nucleotide polymorphisms (SNPs). These genetic variants represent substitutions in which an alternate base pair is present at a particular nucleotide location. The prevalence of SNPs is roughly one in every thousand nucleotides in the human genome. Until recently, use of the term SNP was restricted to polymorphisms present in >1% of the population. However, databases like the dbSNP of the National Center for Biotechnology Information (NCBI), US National Library of Medicine do not use a lower bound on the minor allele frequency in defining what constitutes a SNP. SNPs have been intensively studied in disease susceptibility[18, 19] and pharmacogenetics[20] studies and it is reasonable to hypothesize that SNPs may also affect the induction and processing of damage from ionizing radiation. Two approaches are being pursued to investigate the association of SNPs with the development of adverse normal tissue effects after radiotherapy: candidate gene studies and genome wide association studies (GWAS). Candidate genes are those whose function suggests that they may be mechanistically involved in some aspect of radiation damage induction, repair or damage processing and tissue remodeling. Typically, candidate gene studies concentrate on SNPs causing non-conservative amino-acid changes in the final gene product or SNPs located jn regulatory

regions, possibly affecting gene expression or protein secretion rates. In a recent review, Alsner et al.[21] summarized data from no less than 39 studies, albeit some of them reporting on an extended or different set of SNPs as a previously reported patient series. While a majority of these studies have found encouraging associations between selected SNPs and radiation toxicity, no SNPs have yet been unequivocally established as associated with a specific radiation reaction.

GWAS take a different approach: the association between alleles of different linked SNPs in a population, the so-called linkage disequilibrium, means that a relatively manageable subset of tag SNPs can capture most of the genetic variation in a region. This technology has led to the recent explosion in publication of disease susceptibility studies[22], looking at a quarter or half a million SNPs in thousands of cases and controls. However, although a number of GWAS are in progress in the setting of radiation therapy side effects, so far none of these have been published. What kind of resolution is required on the risk scale? It can be argued[9] that SNPs conveying an odds ratio of less than about 2 are unlikely to be of practical use in modifying radiation therapy, in view of the many other risk factors that have been identified. This means that GWA studies to identify SNPs associated with the development of radiation-induced normal tissue toxicities require fewer subjects (hundreds rather than thousands) to achieve a particular statistical power compared with most disease association studies. An important added advantage of the radiotherapy patient studies is that the environmental agent, ionizing radiation, and the doses to which the subjects are exposed are known. This means, however, that both dosimetric and dose-volume variability must be carefully controlled as these will be confounders in a study of biological associations.

4. Response markers

Response markers are of interest partly because they could serve as individualized in vivo dosimeters for biologically effective dose and partly because they could form the basis for biological adaptive radiotherapy. As an example, the above study from the University of Rochester[17] measured weekly levels of IL-1α and IL-6 during fractionated radiotherapy and tested whether changes in the level of these cytokines 1 to 5 weeks after the start of therapy relative to baseline levels, showed stronger correlation with ultimate outcome than the baseline values themselves. However, the authors concluded that this was not the case.

In clinical studies, a normalization of plasma TGF-β1 levels toward the end of a course of radiotherapy yielded a PPV of 90% for identifying patients who did not develop radiation pneumonitis[23]. This formed the hypothesis underlying a subsequent dose escalation study in 38 patients with inoperable non-small cell lung cancer[24]. This study showed that patients with normalization of TGF-β1 levels toward the end of radiotherapy, could be dose escalated from 73.6 Gy to 80 Gy (8 patients) or 86.4 Gy (6 patients). However, the authors concluded that long-term survivors had a “significant risk” of developing severe treatment-related complications. Evans and colleagues[25] re-assessed the value of TGF-β1 as a response marker and concluded interestingly that this marker was only associated with radiation pneumonitis in a sub-group of patients with unfavorable dose-volume metrics. This illustrates the importance of controlling for dose distribution as a potentially important confounder in this kind of study, particularly when assessing such radiation sensitive organs as the lung.

A further illustration of the concepts introduced here is provided by the recent study by Zhao et al.[26] who did not find an association between radiation-induced lung toxicity (RILT, defined as pneumonitis or fibrosis) and plasma TGF-β1 at baseline in 165 patients with non-small lung cancer. In a subset of 102 cases, plasma TGF-β1 concentration was measured 4 weeks into the course of fractionated radiotherapy. This value was not significantly

associated with the risk of RILT whereas the ratio between the TGF-β1 level during and before RT showed a significant association with subsequent RILT. Using the terminology proposed here, we would conclude that baseline TGF-β1 level is NOT a *predictive factor* for RILT and that the TGF-β1 level during radiotherapy is NOT a *response marker*. However, the change in the TGF-β1 level relative to baseline IS a *response marker* in Zhao's study.

Why distinguish between response markers and predictive markers? There are very basic differences between factors that are given at baseline and that can predict the risk of radiotherapy effects even before the first dose fraction is delivered and biomarkers that are induced by the radiation and therefore are biological "responses" in themselves that may precede a subsequent clinical effect. Genetic differences are obviously not responses, elevated cytokine markers after the start of RT obviously are. The two classes of biomarkers differ in terms of study design methodology, biological significance and potential use in clinical management.

5. Surrogate endpoints for normal tissue effects

Surrogate endpoints for efficacy have attracted considerable interest in drug development trials[27]. Surrogate toxicity endpoints are of great potential interest in radiation oncology trials, especially early surrogates of late radiation effects. An example is the use of increased low grade toxicity as an indicator of increased severe toxicity can be seen as a surrogate endpoint. Although lower-grade toxicity in some cases may progress into higher-grade toxicity[28], this is not obligatory in all cases. The aim of using lower-grade toxicity as a surrogate for higher-grade toxicity is partly to gain a lead time in assessing toxicity but in particular to improve statistical resolution by increasing the number of events. Another example is confluent mucositis after cytotoxic treatment for head and neck cancer, which is a clinical endpoint in its own right, but may also be seen as a useful surrogate endpoint reflecting treatment intensity with respect to other early toxicities. It is correlated with, but not mechanistically related to, the clinical endpoints of pain and dysphagia[29]. Validation of surrogacy requires demonstration of a statistical association between changes in the clinical endpoint and changes in the surrogate endpoint in a population of patients[30]. Functional and molecular imaging data are also examples of biomarkers that are of potential interest as normal tissue response markers or surrogate endpoints (see the paper in this issue by Jeraj et al.).

6. Dose distribution and biomarkers

Modern radiotherapy techniques have deliberately given rise to a range of absorbed doses in non-target tissues. This is in contrast to target volume dose distributions for which the vast majority of current treatment plans prescribe a uniform dose distribution with the aim of delivering this within a relatively narrow tolerance band.

The intricacy of separating dose distribution and treatment intensity from biomarker analysis is illustrated by Evans et al.[25] who noted that plasma TGF-β1 level has been shown to be correlated with mean lung dose (MLD) in patients receiving radiation therapy for lung cancer and that the tumor itself may produce TGF-β1. The observation, that larger tumors are more likely to be treated with plans characterized by a higher MLD, completes the circle. All of this will obviously confound the possible relationship between plasma TGF-β1 level and the clinical incidence of radiation pneumonitis.

Dose-volume metrics and predictive markers add variability in toxicity outcome data that will reduce the statistical power to detect an association between these factors and outcome. Studies are emerging that correct for dosimetric and patient-related risk factors when trying to link SNPs with a clinical phenotype[31, 32]. The candidate gene SNP study from the Cross

Cancer Institute[33] is illustrative in this context. This group looked for an association between 49 SNPs in 24 candidate genes vs. Grade 2+ rectal and bladder toxicity in a series of 83 patients receiving definitive radiotherapy for prostate cancer. Ranked in order of statistical significance the three most significant predictors were: 1) Rectal D_{30} >75 Gy; 2) XRCC3 A>G, 5′ untranslated region 4541; and 3) age at diagnosis ≥ 60 y. Ranked in order of the magnitude of the hazard ratio, the same three factors topped the list, but the order was 1) XRCC3 A>G, 5′ untranslated region 4541; 2) age at diagnosis ≥ 60 y; and 3) Rectal D_{30} >75 Gy. In a multivariate Cox proportional hazards model, age at diagnosis and D_{30} were included in each of the best and second best subsets of four and five predictors, while the XRCC3 A>G SNP was not selected for inclusion in any of these four models. One or two among a set of four other SNPs were included in at least one of the four models; one of these, ERCC2 G>A, $Asp^{711}Asp$ was non-significant (P=0.08) in univariate analysis but was significant (P=0.02) in a model including age at diagnosis, mean bladder dose, rectal D_{30} and the LIG 4 T>C, $Asp^{568}Asp$ SNP. While the in- or exclusion of specific covariates in Cox models using stepwise selection should be interpreted with great care, these data do illustrate how SNPs selected without adjustment for dose-volume metrics can be selected under a ‘wrong’ model. It is also worth noting that the dose-volume metrics appeared more robust as an explanatory variable in this particular study. From the perspective of estimating dose-volume relationships, the rectal D_{30} showed greater significance and a larger hazard ratio in two of the four models adjusting for selected SNPs. Caveats in the Cross Cancer Institute are the large number of SNPs studied in a relatively small number of patients, increasing the risk of spurious false positive findings, and the decision to pool rectal and bladder toxicities in the analysis. While it may be a reasonable assumption to test whether these depend on common SNPs, the relevant dose-volume metrics for the two organs will not generally be the same. It should also be noted that the plan is only a surrogate of what was actually delivered and ultimately the most valid dose-distribution would be a cumulative one comprising the actual dose from every fraction. That type of data is only starting to be collected on a small scale, see the paper in this issue by Jaffray et al. Despite these limitations, the Canadian study provides an interesting example of the interplay between dosimetric and biological risk factors. These relationships should clearly be explored in large independent studies.

7. Bioinformatics and biomarkers

Findings reported in the literature are often inconsistent across studies. An obvious problem is that most studies have modest sample sizes in relation to the realistic magnitude of likely effect sizes. Other reasons for conflicting findings between studies are a high likelihood of false positive findings due to (1) multiple comparisons, i.e. high dimensionality of the genomic data, (2) the testing of data-generated hypotheses in the same data set, and (3) overfitting, i.e. the use of too many covariates in a predictive model relative to the number of events being analyzed. All of these issues can lead to false associations and reduce the generalizability of the findings to independent data sets[15]. A number of strategies have been proposed to reduce these problems[34] including improved study reporting, assay quality assurance, high-precision dosimetry and improved toxicity scoring[15]. Prospectively planned, independent training and test data sets with large sample sizes will be required to achieve a balance between discovering novel associations on one hand and reducing the false-positive rate on the other[15, 35].

8. Research priorities

As mentioned above, dose distribution and biomarkers are mutually confounding factors in many of the studies conducted to date. From a QUANTEC perspective adjusting for biological and patient-related factors will lead to stronger dose-volume-effect relationships.

From a biomarker discovery perspective, adjustment for dose-volume effects will improve the statistical power to detect biological predictive factors with a specific effect size. Discovery of response markers would typically involve mechanistic studies in patients, who develop a specific radiation effect, but also in this case an understanding of the relationship between local and organ-level effects would typically have to be established. Likewise one first screen for surrogate toxicity endpoints could be to prove dose-response and volume-response sensitivity. Several national and international bio-banks have been set up specifically aimed at association studies with radiation therapy effects[36]. Much has been learned from the first generation of radiogenomics studies. Moving on to the second generation of studies, we propose the following priorities:

1. Large prospective studies to identify associations between specific radiation effect endpoints and candidate predictive markers or GWA studies with careful adjustment for dose-volume relationships and other risk factors. Standardized scoring of the grade of reaction with adequate follow-up time in case of late effects and prospective storing of the full 3D dose matrix should be required from all participating centers. Such trials are under consideration within the Radiation Therapy Oncology Group (RTOG).
2. Training set/validation set designs with prospective validation in truly independent populations.
3. High throughput methodology for mass screening of potential predictive factors, response markers and surrogate endpoints.
4. Discovery of surrogate endpoints of late side-effects to be used in early clinical trials of new combined modality therapies.
5. Development of data analysis strategies that allow correction for the confounding effects of dose distribution as well as patient and treatment related risk factors.

One of the keys to success in the coming five years is to expand the number of cases in normal-tissue effect biomarker studies. This is likely to require large collaborative networks of investigators or studies within the RTOG or other multi-center groups. At the same time, a more rigorous application of training/validation set designs will be required to address the problem of over-fitting and high false-positive rates.

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