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Overview

Biomarkers of Radiation Exposure: Can They Predict Normal Tissue Radiosensitivity?

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Abstract

Late adverse tissue reactions affect up to a fifth of cancer patients receiving radiotherapy, with several clinical parameters known to influence normal tissue responses. Despite careful control of treatment-related parameters, a significant component of inter-individual variability in normal tissue responses remains unaccounted for, suggesting that perhaps intrinsic genetic and epigenetic factors are the major determinants of normal tissue effects. Against this background, research was initiated into cellular markers predictive of clinical radiosensitivity, focusing first on colony-forming assays, before the advent of reliable surrogate end points, such as chromosomal radiosensitivity and DNA damage repair. More recently, collaborative efforts have focused on genotyping analysis at a target gene or whole genome level. Despite early positive reports from several small-scale pilot studies testing these assays, subsequent attempts to reproduce comparable levels of association between the cellular markers and clinical phenotype in larger cohorts have frequently been inconclusive, although the first well-replicated studies are beginning to emerge. Here, we discuss the underlying rationale, consider aspects pertaining to patient recruitment and study design, review some of the reported findings for DNA damage-related markers, and highlight some of the limitations and confounding factors affecting tests of association between predictive markers and clinical radiosensitivity. We propose that an integrative approach incorporating multiple assays involving collaborations across centres, together with prospective meticulous recruitment of patients taking into account modifying clinical factors of normal tissue responses, enhances the chance of finding the long sought after markers of individual radiosensitivity.

Key words: Chromosome aberrations; DNA damage response; gamma-H2AX foci; normal tissue damage; radiosensitivity; radiotherapy

Statement of Search Strategies Used and Sources of Information

A systematic literature search was carried out using Medline up to 5 February 2013 using the key words: 'normal tissue*', 'radiotherapy', 'predictive' and 'marker*'. The search also included the reference list for these articles and selected additional articles and web pages that were judged to be relevant.

Introduction

At first glance, the idea of having an assay capable of estimating normal tissue radiosensitivity in a specific patient seems highly appealing. Intuitively, 'clinical advantages' of a predictive assay of normal tissue radiosensitivity would include exploiting the benefits of radiotherapy dose escalation among the non-radiosensitive patient population, while subjecting the sensitive tail of the normal distribution of intrinsic radiosensitivities to the conventional or even reduced doses. Such a strategy should, in theory, maintain the overall incidence of normal tissue toxicities and improve the therapeutic ratio [1]. In the same vein, other proposed approaches include: (1) screening for rare individuals with extreme radiosensitivity, in order to treat these cases with a reduced total dose [2,3] and (2) individualising radiotherapy doses based on the derived *in vitro*

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cellular radiosensitivity of each individual such that patients receive an isoeffective treatment with equal likelihood of normal tissue toxicities [4]. Judging from these proposals, the clinical benefits of such an assay, if it exists, are self-evident, and for decades the search for a robust predictive assay of normal tissue radiosensitivity has been the subject of intensive research within the radiotherapy community.

The early assays tested for their predictive value of normal tissue responses were primarily colony-forming assays carried out mostly in fibroblasts or lymphocytes isolated from skin biopsies or peripheral blood samples of patients, respectively. There is a reasonable amount of evidence to suggest that *in vitro* cellular radiosensitivities of these cell types are indicative of *in vivo* normal tissue responses after radiotherapy [5–11]. However, cellular clonogenicity testing is slow, labour intensive and requires considerable technical expertise, rendering it unsuitable for routine clinical use. This led to the testing of alternative cellular end points that may be reproducible surrogates of loss of clonogenicity. Chromosome aberrations and DNA damage, specifically DNA double-strand breaks (DSB), are among the few cellular markers that have shown a certain level of correlation with *in vitro* cellular radiosensitivity and *in vivo* normal tissue responses [12–28].

However, the considerable efforts have been rewarded with only limited success, often confined to small case–control pilot studies. Typically, a promising predictive marker of normal tissue radiosensitivity, proposed on the basis of correlation between cellular and clinical end points within a small cohort of radiotherapy patients, would fail to reproduce comparable levels of association when tested in larger and more heterogeneous patient cohorts [5,6,9,29,30]. Other times, conflicting results would arise from independent studies investigating the same predictive assay of normal tissue radiosensitivity, thus questioning the reproducibility and reliability of the tested assay [22,31,32]. Importantly, the potential to establish a correlation between a cellular marker and clinical radiosensitivity is heavily influenced by a number of factors [33]. Briefly, these factors relate to the selection of 'cases' and 'controls' while ensuring that modifying factors of normal tissue responses are considered, adopting an appropriate clinical end point as an indicator of clinical radiosensitivity, quality and consistency of clinical follow-up, using an assay with a well-established laboratory protocol, together with choosing the optimal experimental conditions for test dose, dose rate, time point, and cell type.

Clinical Considerations in the Selection of 'Over-responders' and 'Controls'

The ability to show an association between the results of an *in vitro* radiosensitivity assay and the degree of normal tissue damage relies significantly on the accurate and objective assessment of the clinical phenotype. Although it may not be possible to fully ascertain whether the clinical severity of normal tissue reactions truly reflects the intrinsic radiosensitivity of an individual, nonetheless, a wide range of clinical parameters known to influence normal tissue responses has to be considered during patient stratification. These include radiation dose, fraction size, anatomical features and additional treatments such as systemic therapy and surgery. In addition, the duration of follow-up could also affect the relative relationship between cellular and clinical end points. Based on the work by Jung and colleagues [34], it is well characterised that the incidence of late effects in several organs occurs with exponential kinetics, and in some individuals these effects may only manifest years after radiotherapy [34]. Conversely, individuals presenting early on with late effects do not necessarily imply clinical radiosensitivity. On this basis, it may be prudent to test the chosen cohorts of 'over-responders' and 'controls' for their rates of developing late tissue effects using the log-linear plots proposed by Jung *et al.* [34] to confirm that they are indeed phenotypically distinct, before testing predictive markers.

Ideally, investigations into the efficacy of an *in vitro* cellular assay to predict normal tissue radiosensitivity should preferably include homogenously treated patients with accurate documentation of treatment-related parameters relevant to the normal tissue of interest. Assessment of the clinical end point should be carried out using a well-established method of scoring and the employed system should be adequately sensitive to allow for the detection of significant variation between individuals [35]. For reasons provided above, patients should also have consistent follow-up periods to ensure that their clinical ranking corresponds to the assigned phenotype [36]. Lastly, factors, other than the cellular parameter being tested, that are known to influence normal tissue responses should be incorporated into a multivariate analysis. In reality, this is often not feasible considering the small scale of these studies.

Controversies of Case–Control Studies Testing for Predictive Markers of Normal Tissue Radiosensitivity

The application of a case–control study design for the testing of predictive markers of normal tissue radiosensitivity has also been called into question. Most notably, in a 2003 editorial by Dikomey and colleagues [36], basing their analysis on data reported by Peacock *et al.* [30], it was elegantly shown that although comparative analysis of mean cellular radiosensitivity between cases and controls did not reveal a difference, a positive association between cellular radiosensitivity and late clinical effects may have been observed in that study had the authors undertaken a different approach for data analysis. This approach is based on the assumption that late normal tissue effects occur at a constant annual rate in radiotherapy patients across all spectra of intrinsic radiosensitivity, as suggested by the work of Jung *et al.* [34]. Assuming that 'resistant' patients are also liable to severe late effects of radiotherapy, it would be unsurprising why the comparison of mean cellular

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