



Lucy Kettlewell. *Boats, Michigan #4*. Oil on board. From the personal collection of John Horton.

An accurate radiosensitivity assay is central to the development of personalized treatment strategies. Predicting tumor radiosensitivity is possible, but further developments are required before an accurate assay can be routinely applied.

Predicting Response to Clinical Radiotherapy: Past, Present, and Future Directions

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Background: Personalized radiation therapy holds the promise that the diagnosis, prevention, and treatment of cancer will be based on individual assessment of risk. Although advances in personalized radiation therapy have been achieved, the biological parameters that define individual radiosensitivity remain unclear:

Methods: This review focuses on discussing the field of radiosensitivity predictive assays, a technology central to the concept of personalized medicine in radiation oncology. Two novel approaches, DNA end-binding complexes and gene expression classifiers, show promise in solving some of the logistic problems associated with previous assays.

Results: Current data suggest that predicting clinical response to radiotherapy is possible. The delivery of this promise depends on the ability to define the variables that define response to clinical radiotherapy. A successful predictive assay is key to the development of personalized treatment strategies in radiation oncology.

Conclusions: Novel technologies need to be developed that will improve our understanding of the biological variables that define clinical tumor response and will lead to the development of a clinically useful assay.

Introduction

Since it was first shown to be effective over 100 years ago, ionizing radiation has been a central player in the treatment of malignancies. Its therapeutic role has evolved over the last century as technologic advances have improved the ability to deliver high doses of radiation to tumor volumes while avoiding normal tissues.

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Abbreviations used in this paper: Tpot = tumor proliferative potential, HIF = hypoxia-inducible factor, SF2 = survival fraction at 2 Gy.

The individualization of radiation therapy is hardly a new concept. Over the last two decades, significant technologic refinements resulting from the integration of 3-D anatomy into radiation treatment planning systems have led to the individualization of treatment fields. Thus, radiation treatment portals are personalized based on individual normal tissue and tumor anatomy. Further strides in individualized radiation therapy have been achieved with the development of intensity-modulated radiation therapy (IMRT) techniques and image-guided (IGRT) techniques.¹

Although important advances toward personalized radiation therapy have been achieved largely by physical advances in radiotherapy planning and delivery, the efforts in understanding the biological parameters that define individual radiosensitivity have not achieved

the same success. Thus, radiotherapy is prescribed without considering the potential individual differences in tumor and patient radiosensitivity. However, there is evidence to suggest that individual differences in inherent radiosensitivity do exist and thus understanding their biological basis could impact significantly how clinical radiation oncology is practiced. Further, this knowledge could be exploited to develop an assay to predict clinical radiation response at the individual level.

Development of Predictive Assays

The development of a successful clinical assay to predict response to radiation therapy is a major clinical goal in radiation oncology.^{2,3} The clinical impact of an accurate assay would be broad and significant since approximately 60% of cancer patients are treated with radiation therapy.⁴ For example, such technology could result in better selection of patients for radiotherapy protocols, could improve assessment of individual response and prognosis, and could lead to the personalization of radiation dose parameters. Thus, it is no surprise that Gilbert H. Fletcher, MD, compared the development of a successful radiotherapy predictive assay to the quest for the Holy Grail.²

In general, the variables determining radiotherapy response can be grouped into three different categories: (1) intrinsic radiosensitivity, (2) tumor oxygenation status, and (3) tumor proliferative potential (Tpot). Clinical approaches to address each of these categories have been developed and tested as clinical predictors of radiotherapy response: (1) determining ex vivo tumor SF2 (survival fraction at 2 Gy) using clonogenic survival assays, (2) utilizing electrodes to measure tumor oxygenation status, and (3) determining Tpot.³

Determination of Tumor Inherent Radiosensitivity

The clonogenic cell survival assay has been the gold standard to measure cellular response to radiotherapy in the laboratory. However, its clinical application has been hindered because of the technical difficulties of plating tumor cells ex vivo. In spite of this, some data suggest that clinical response differences do exist based on tumor intrinsic radiosensitivity. West et al⁵⁻⁷ reported the largest study to date examining SF2 as a predictive/prognostic factor in cervical cancer. In their series, SF2 was the most important variable correlated with clinical outcome. The 5-year survival rate for patients with radiosensitive tumors (SF2 <0.42) was 81% compared with 51% for patients with radioresistant tumors (SF2 >0.42). Björk-Eriksson et al⁸ reported a similar finding in 99 head and neck cancer patients where a superior clinical outcome was correlated with an SF2 below 0.40. In contrast, other authors have not found a correlation between ex vivo SF2 and clinical outcome (Table).⁹⁻¹¹ This assay is technically difficult as ex vivo tumors have about a 1% plating efficiency. Furthermore, the result of the assay is not available at the time of treatment decision since plating and generating the clonogenic assays can take several weeks. Furthermore, in the series by Björk-Eriksson et al,⁸ successful SF2 assays were not generated in 30% of patients because colonies did not grow. Therefore, it could be argued that the technical difficulties involved in assay standardization and optimization significantly interfered with the ability to further study the predictive accuracy of the assay. If a more practical approach can be developed, further study of intrinsic radiosensitivity as a determinant of clinical response to radiotherapy is warranted.

Table. — SF2 as a Correlate of Clinical Response

| Disease Site | No. of Patients | SF2 Cutpoint | Outcome | Positive Study | Reference |
|-------------------------|-----------------|----------------|---|----------------|-----------------------------------|
| Head and Neck | 99 | 0.4 | Local control <0.4 vs >0.4 91% vs 74% P=.036 | Yes | Björk-Eriksson et al ⁸ |
| Cervix | 128 | 0.42 | Survival <0.42 vs >0.42 81% vs 51% P=.0002 | Yes | West et al ⁶ |
| Head and Neck | 38 | 0.5 | Local control <0.5 vs >0.5 26% vs 45% P=NS | No | Staubøl-Grøn et al ⁹ |
| Glioblastoma multiforme | 50 | Not determined | No correlation between SF2 and survival | No | Taghian et al ¹⁰ |
| Head and Neck | 92 | Not determined | No correlation between SF2 and survival | No | Eschwege et al ¹¹ |

Tumor Oxygen Status and Clinical Response

The correlation between radiosensitivity and the presence of oxygen, sometimes referred to as the “oxygen effect,” was first recognized in the early days of radiation biology. Thus, determining tumor oxygen status has been an area of focus for predictive assay development. Oxygen probes have been clinically applied to this question, with the Eppendorf probe probably being the most successful. Movsas et al¹² measured PO₂ in 57 prostate cancer patients treated with brachytherapy. They obtained measurements from the prostate area where cancer had been detected and from normal muscle that they used for normalization. They found that 2-year biochemical control was statistically different for patients with a hypoxic prostate/muscle PO₂ ratio of less than 0.05 (31% vs 92%, $P<.0001$). The statistical significance of this ratio held on multivariate analysis.

Hypoxia as a predictor of radiation response has also been studied in cervical cancer. In a prospective validation trial in 106 patients with cervical cancer, Fyles et al¹³ showed that hypoxic tumor had a worse 3-year progression-free survival rate when compared to better oxygenated tumors (37% vs 67%, $P=.004$). However, as the clonogenic assay, this approach has been limited by its practicality. For example, it is an invasive test that is easy to apply in readily accessible tumors (eg, head and neck, cervix) but is impractical for deep-seated tumors. To address these practical issues, several studies have looked at hypoxia-inducible factor 1 α (HIF-1 α) as a surrogate of tumor hypoxia. Bachtary et al¹⁴ initially reported on HIF-1 α expression and clinical outcome in 67 patients with cervical cancer treated with radiotherapy. They concluded that HIF-1 α had both predictive and prognostic abilities in these patients. In contrast, Hutchison et al¹⁵ could not confirm these results in 99 patients with cervical cancer.

Tpot as a Predictor of Response to Radiotherapy

Tumor repopulation between treatment radiation fractions is an important mechanism responsible for treatment failures after radiotherapy. To address this, altered fractionation schemes have been studied as an approach to impact interfraction tumor repopulation. Fu et al¹⁶ reported a prospective phase III randomized trial in 1,073 head and neck cancer patients that showed a statistically significant improvement in local control achieved by accelerated and hyperfractionated radiotherapy over standard fractionated radiotherapy. Thus, clinical evidence supports tumor repopulation as an important failure mechanism in at least a subset of patients.

Tpot is an assay designed to determine the potential doubling time of a tumor. This calculation is derived from a tumor biopsy stained with bromodeoxyuridine and analyzed via flow cytometry. This parameter was

tested in a multicenter analysis by the European Organization for Research and Treatment of Cancer (EORTC) of 476 head and neck cancer patients and was not a predictor of outcome.¹⁷ Furthermore, a similar result was reported by Bourhis et al,¹⁸ where no advantage in local control was found in patients with longer Tpot (mean Tpot local failures vs local controls, 5.3 days vs 6.1 days, no statistical difference). In contrast, Corvó et al¹⁹ found that Tpot correlated with local control in 37 head and neck patients treated with conventional radiotherapy (2-year local control, Tpot >5 days vs Tpot \leq 5 days, 42% vs 8%, $P=.0006$). Unfortunately, this initial promising result was not confirmed by the large multicenter EORTC analysis. Thus, although tumor repopulation appears to be responsible for at least some of the failures following radiotherapy, Tpot is at best a weak predictor of outcome.

Correlation of DNA End-Binding Complexes With Cellular Radiosensitivity

DNA double-strand breaks are thought to be the lethal lesion caused by radiation.²⁰⁻²² DNA damage induces a cellular response that includes activation of a number of signal transduction cascades. These include ataxia telangiectasia mutant (ATM) and DNA-dependent protein kinase pathways (DNA-PK) that, among other things, allow time for DNA repair.²³⁻²⁶ Thus, the ability to repair radiation-induced damage is critical in determining intrinsic radiosensitivity. In order to exploit this, Ismail et al²⁷ developed an assay to analyze DNA end-binding complexes. They identified a rapidly migrating ATM-containing band (B and A) of which the density correlated with radiosensitivity in both primary fibroblasts and cancer cell lines. Fig 1 shows the linear correlation between B and A density and radiosensitivity.

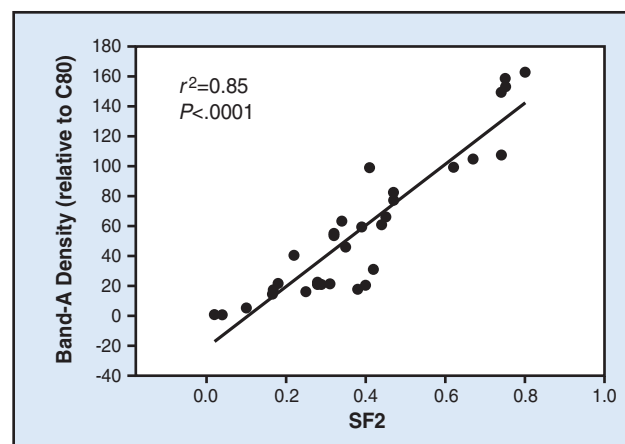


Fig 1. — Band-A density correlated with SF2. Densitometry was performed on band-A (in triplicate, from different extracts on different gels) from 36 primary and tumor cell lines. Mean SF2 was correlated with mean band-A density ($r^2=0.85$). From Ismail SM, Puppi M, Prithivirajsingh S, et al. Predicting radiosensitivity using DNA end-binding complex analysis. *Clin Cancer Res*. 2004;10:1226-1234. Copyright 2004 by the American Cancer Society (ACS). Reproduced with permission of the ACS via Copyright Clearance Center.

ty in primary fibroblasts and cancer cell lines. Although this approach is yet to be translated into the clinic, it potentially solves some of the practical issues associated with previous assays since it can be performed from tumor biopsies and interpreted before the initiation of therapy. We believe initial testing of this concept in the clinic is justified.

Prediction of Radiosensitivity Using a Gene Expression Classifier

One of the common themes that unify all mechanisms discussed above is that they all induce changes in gene expression. Hypoxia induces genetic change through upregulation of HIF-1 α .²⁸ Genes that are transcriptionally activated by HIF-1 α include genes involved in proliferation (cyclin G2, IGF2, TGF, WAF1, etc), cell survival (ADM, EPO, VEGF), apoptosis (NIX, NIP3), angiogenesis (TGF- β 3, VEGF), and other important cellular processes.²⁸ Finally, several genetic factors have been correlated with cellular radiosensitivity. Among these, RAS^{29,30} and ATM³¹ have been correlated with radioresistance and radiosensitivity, respectively.³¹ Other inherited human syndromes that have been associated with sensitivity to radiation include basal cell nevoid syndrome, Cockayne's

syndrome, Down syndrome, Fanconi's anemia, and Gardner's syndrome. Therefore it is reasonable to conclude that differences in gene expression may detect differences in radiation phenotype.

In preliminary studies, a gene expression classifier was developed to predict cellular radiosensitivity in 35 cancer cell lines.³² The algorithm scheme is shown in Fig 2. Radiosensitivity was defined as SF2 and treated as a continuous variable. A leave-one-out cross validation (LOOCV) approach was utilized where the classifier was developed using 34 of the 35 cell lines as a training set, leaving one cell line as a test set. A linear regression plot of gene expression vs SF2 was generated for each gene in the database (7,136 transcripts). Genes were ranked by linear fit and the genes with the best linear fit were used to develop the predictive tool. A multivariate predictor was then generated in each round of LOOCV and tested with the held-out sample. A correct prediction was called if the predicted SF2 was within 10% of the measured SF2. After 35 rounds of this process (each round the test set is a different cell line), an accuracy of 62% was achieved, which is statistically different from chance ($P=0.002$). Importantly, they tested the biological validity of the algorithm by demonstrating that some

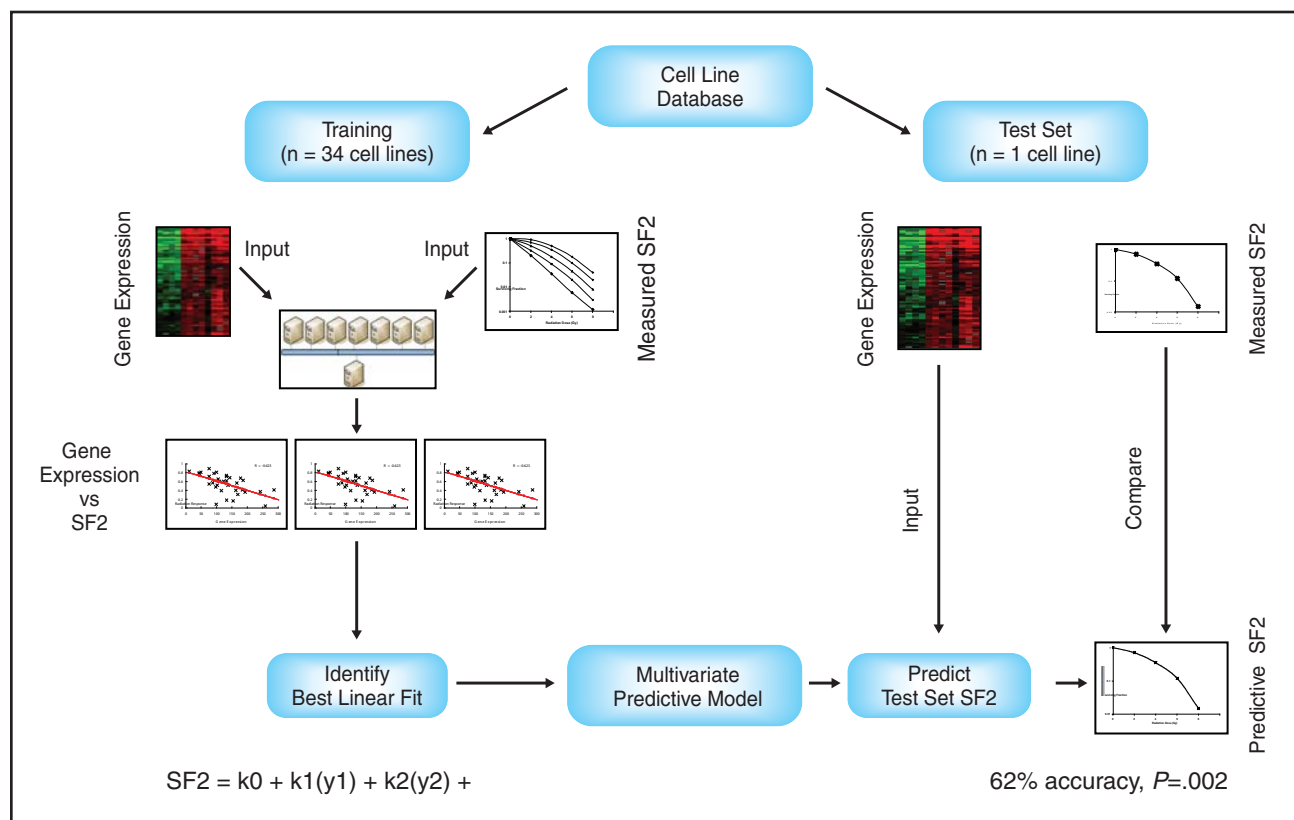


Fig 2. — Algorithm for radiation sensitivity gene expression classifier. Linear regression is used to screen a training database of 34 cancer cell lines for correlations between gene expression and radiosensitivity (SF2). The genes with best linear fit are identified to build a multivariate linear regression model represented by the equation $SF2 = k_0 + k_1(y_1) + k_2(y_2)$ where y is the gene expression of the selected genes. The test based on SF2 as a continuous variable showed a 62% accuracy which was statistically significant when compared to chance. In 10,000 permutations of chance prediction, only two times chance was as accurate as the classifier. From Torres-Roca JF, Eschrich S, Zhao H, et al. Prediction of radiation sensitivity using a gene expression classifier. *Cancer Res.* 2005;65:7169-7176. Copyright 2005 by American Association for Cancer Research (AACR). Reproduced with permission of AACR via Copyright Clearance Center.

of the genes identified were mechanistically involved in radiation response. Therefore, these experiments establish that radiosensitivity is predictable based on gene expression and open the possibility that genomic-based approaches may result in clinically applicable radiosensitivity molecular signatures.

Several molecular signatures have been described in cervical and rectal cancer. Watanabe et al³³ reported a 33 gene set that discriminated between responders and nonresponders in a cohort of 52 rectal cancer patients treated with preoperative radiotherapy. Also, Wong et al³⁴ showed that using 10 genes, neural networks could distinguish between clinically radioresistant and radiosensitivity tumors in 16 patients with cervical cancer. Although promising, these initial results await further validation in larger and independent clinical subsets. Furthermore, besides leading to predictive/prognostic signatures, profiling experiments could identify novel molecular targets for drug development that could lead to the development of targeted radiosensitizers/radioprotectors. The success of cetuximab in head and neck cancer provides a rationale for further development of targeted radiosensitizers.³⁵

Gene expression classifiers to predict normal tissue complications have also been developed. Svensson et al³⁶ described a classifier to predict toxicity in prostate cancer patients treated with radiation therapy. In their approach, they generated gene expression profiles from lymphocytes 24 hours after mock or 2 Gy of irradiation. They identified several gene sets that discriminated between patients with and without late radiation toxicity. Their overall classifying accuracy in a binary classification problem was 86%, although less accurate at the individual level (55%). In another approach, Rødningen et al³⁷ generated gene expression profiles from fibroblast cell lines developed from breast cancer individuals with variable risk for radiation-induced fibrosis. Fibroblasts were treated with two different radiation schemes: 3.5 Gy in one fraction with RNA isolated 2 hours and 24 hours after irradiation or with a fractionated scheme 10.5 Gy in three fractions with RNA isolated 24 hours after the last fraction. Thus in both approaches, toxicity was being correlated to radioresponsive genes. An accurate classifier with a minimum set of 18 genes was developed that correctly classified 12 patients. Although both of these approaches require further validation, both studies support the idea that inherent radiosensitivity may play a role in determining individual risk for radiation toxicity.

Conclusions

Although significant efforts have been directed at developing an assay to predict clinical response to radiotherapy, none of the approaches developed have become routine in the clinic. However, the data suggest that predicting clinical response to radiotherapy is pos-

sible. The two novel approaches (DNA end-binding complexes and gene expression classifier) discussed above might solve some of the logistic problems associated with previous assays that prevented their testing in a larger clinical population. Currently, both are being translated into the clinic. Although a practical and accurate assay continues to elude us, the importance of such technology is highlighted in the era of personalized therapy. We think it is likely that novel omic-based technologies will improve our understanding of the biological variables that define clinical tumor response and may lead to the development of a successful assay.

Disclosures

No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

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