Precision Medicine and Imaging

Clinical Cancer Research

Integrating Radiosensitivity and Immune Gene Signatures for Predicting Benefit of Radiotherapy in Breast Cancer

Yi Cui, Bailiang Li, Erqi L. Pollom, Kathleen C. Horst, and Ruijiang Li

Abstract

Purpose: Breast cancer is a heterogeneous disease and not all patients respond equally to adjuvant radiotherapy. Predictive biomarkers are needed to select patients who will benefit from the treatment and spare others the toxicity and burden of radiation.

Experimental Design: We first trained and tested an intrinsic radiosensitivity gene signature to predict local recurrence after radiotherapy in three cohorts of 948 patients. Next, we developed an antigen processing and presentation-based immune signature by maximizing the treatment interaction effect in 129 patients. To test their predictive value, we matched patients treated with or without radiotherapy in an independent validation cohort for clinicopathologic factors including age, ER status, HER2 status, stage, hormone-therapy, chemotherapy, and surgery. Disease-specific survival (DSS) was the primary endpoint.

Results: Our validation cohort consisted of 1,439 patients. After matching and stratification by the radiosensitivity signature, patients who received radiotherapy had better DSS than patients who did not in the radiation-sensitive group [hazard ratio (HR), 0.68; $P = 0.059$; $n = 322$], whereas a reverse trend was observed in the radiation-resistant group (HR, 1.53; $P = 0.059$; $n = 202$). Similarly, patients treated with radiotherapy had significantly better DSS in the immune-effective group (HR, 0.46; $P = 0.0076$; $n = 180$), with no difference in DSS in the immune-defective group (HR, 1.27; $P = 0.16$; $n = 348$). Both signatures were predictive of radiotherapy benefit ($P_{\text{interaction}} = 0.007$ and 0.005). Integration of radiosensitivity and immune signatures further stratified patients into three groups with differential outcomes for those treated with or without radiotherapy ($P_{\text{interaction}} = 0.003$).

Conclusions: The proposed signatures have the potential to select patients who are most likely to benefit from radiotherapy. *Clin Cancer Res; 24(19); 4754–62. ©2018 AACR.*

Introduction

Radiotherapy is an integral component in the treatment of breast cancer. Two large meta-analyses have shown that adjuvant radiotherapy reduces local recurrence and improves survival after breast-conserving surgery (1) and mastectomy (2). However, breast cancer is increasingly recognized as a heterogeneous disease and not all patients derive survival benefit from radiotherapy, nor is the absolute benefit equal across risk groups (3). With local recurrence rates in breast cancer declining due to improvements in screening, pathologic examination, and modern systemic therapy, it is essential to identify patients who may not benefit from radiotherapy and who may avoid the morbidity and burden of adjuvant radiotherapy (4).

Gene-expression signatures are useful tools that may allow clinicians to tailor therapies according to the molecular characteristics of individual tumors (5). For instance, the 21-gene Oncotype DX signature is used to estimate risk of distant recurrence (ref. 6; i.e., prognostic) and identify patients who would benefit from adjuvant chemotherapy (refs. 7, 8; i.e., predictive) in early-stage breast cancer. No such gene signature has been prospectively validated in randomized controlled trials to inform adjuvant radiotherapy (9). Most existing signatures are prognostic of local recurrence but few have been shown to be predictive of radiotherapy benefit (10–16).

We aimed to develop gene signatures by integrating two distinct biological processes for predicting benefit of radiotherapy in breast cancer. Accumulating evidence supports that therapeutic effects of radiation are influenced and modulated by the tumor microenvironment, including the immune system (17), which recognizes cancer cells via the presentation of tumor-associated antigens on the major histocompatibility complex (MHC) molecules (18). Therefore, we hypothesized that, in addition to tumor intrinsic radiosensitivity, differences in antigen processing and presentation (APP) may lead to differential immune-mediated antitumor response activated by radiation and thus could correlate with clinical response to radiotherapy. We validated the gene signatures in an independent retrospective cohort with long-term clinical follow-up, where patients are balanced for clinical and treatment characteristics using a matched strategy.

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Translational Relevance

Radiotherapy is a mainstay in the treatment of breast cancer and has been shown to reduce local recurrence and improve survival. However, breast cancer is increasingly recognized as a heterogeneous disease and not all patients derive survival benefit from radiotherapy. In this work, we developed gene-expression signatures based on two distinct biologic processes, that is, intrinsic radiosensitivity and antitumor immunity. Both signatures were significantly associated with differential outcomes of breast cancer depending on whether patients had received radiotherapy or not, demonstrating their potential predictive value. Integration of the two signatures further improved patient stratification. We envision that the proposed signatures, if validated in prospective randomized trials, may be used to select patients with breast cancer who are most likely to benefit from adjuvant radiotherapy.

Materials and Methods

Study design and patients

This study was approved by institutional review board (IRB) and conducted in accordance with ethical guidelines, including the Declaration of Helsinki and Belmont Report. Patient informed consent was waived given the use of existing, de-identified public datasets. We developed two gene expression–based signatures, that is, a radiosensitivity signature (RSS) and an immune signature (IMS), and independently validated their predictive value in an external cohort using public gene expression profiles of fresh-frozen tumors (Fig. 1). To train and validate RSS, we searched for publicly available breast cancer gene-expression datasets where all patients received radiotherapy, and selected three largest datasets. Specifically, we trained the RSS using a cohort of 343 patients with invasive breast cancer treated with breast-conserving surgery (BCS) and radiotherapy, who had at least 10 years follow-up (19). Microarray gene expression data were available from Gene Expression Omnibus (accession number GSE30682). Local recurrence-free survival (LRFS) was the clinical endpoint for training purposes. The prognostic value of the RSS was assessed in two independent cohorts of 319 patients (refs. 5, 20; NKI dataset) and 286 patients (ref. 21; accession number GSE2034). All the patients in the NKI cohort received radiotherapy with a median follow-up time of 7.1 years (range, 0.05–18.4). For the GSE2034 cohort, the majority of the patients (87%, $n = 248$) received radiotherapy, and since patient-level radiation treatment information was not available, validation was performed in the entire cohort. For these two validation cohorts, relapse-free survival (RFS) was used as the endpoint because information about LRFS was not available.

To develop a predictive immune gene signature, we searched for breast cancer gene expression datasets in which individual patient-level information about radiotherapy treatment and clinical outcomes was available. The three largest cohorts were the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort, The Cancer Genome Atlas (TCGA) breast cancer cohort, and the invasive breast cancer cohort (E-TABM-159) of Chin and colleagues (22). TCGA cohort was not used due to relatively short follow-up times. We trained an immune signature using the Chin cohort (E-TABM-158) in which 66 patients received adjuvant radiotherapy and 63 did not. The median follow-up time was 6.0 years (range: 0.13–14.2). Disease-specific survival (DSS) was the clinical endpoint for training the immune signature.

The two signatures (RSS and IMS) were evaluated for their ability to predict the benefit of radiotherapy in the METABRIC

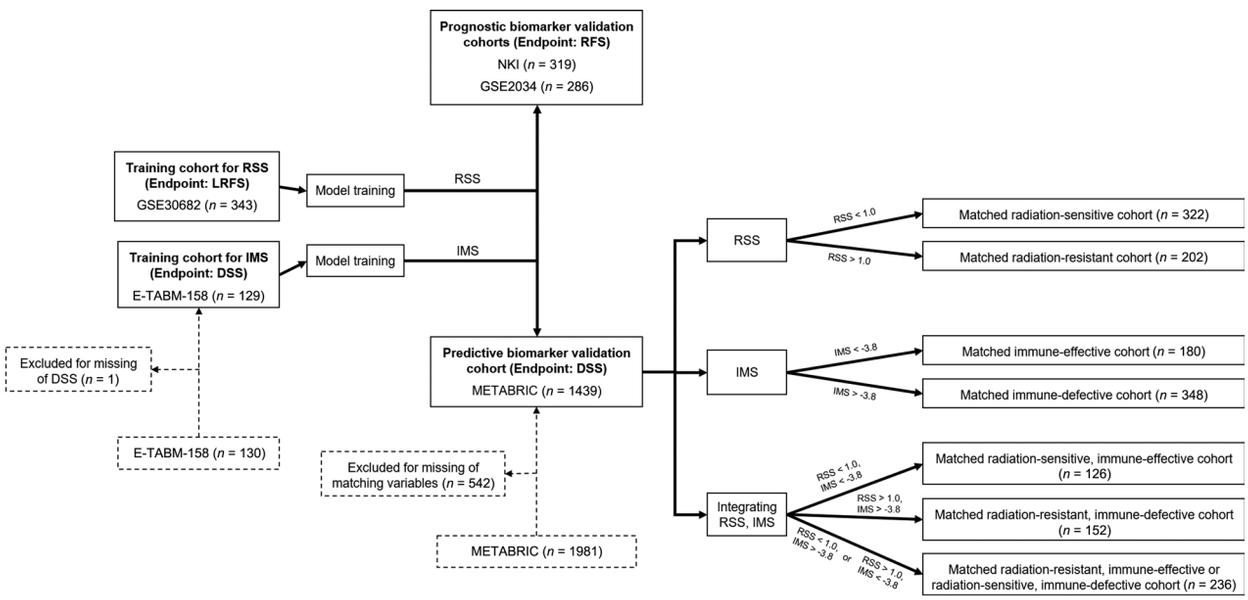

Figure 1.

Schema of study design and patient selection. RSS and IMS were independently developed. Their predictive value was assessed using patients in METABRIC who were matched within each biomarker group. The prognostic value of RSS was assessed in two additional independent cohorts. RSS: radiosensitivity signature; IMS: immune signature; LRFS: local recurrence-free survival; RFS: relapse-free survival; DSS: disease-specific survival.

cohort, which contains gene-expression profiles and outcomes for 1,981 patients with a median follow-up time of 9.7 years (range, 0.0–29.6; ref. 23). To minimize selection bias and confounding effects, we used a matched strategy to balance patients in each biomarker-defined group. First, the validation cohort was dichotomized by a predefined cutoff value according to respective gene signatures. Then within each subgroup, we performed exact 1:1 matching of patients who received radiotherapy versus those who did not according to seven clinical and treatment characteristics. The matching variables were age (< or ≥50 years), ER status, HER2 status, stage, hormone therapy, chemotherapy, and surgery type (lumpectomy or mastectomy; Data Supplementary). Predictive value was assessed using the appropriate matched patient cohorts. DSS was the clinical endpoint.

Development of RSS and IMS

To train the RSS, we first compiled a list of radiation-related genes by searching for gene sets whose name contains "RADIATION" or "IR" in MSigDB (24). This initial gene list was further refined by excluding genes that are also present in the immune-related gene sets in ImmPort (25). Next, we performed univariate Cox regression analysis and identified genes significantly associated with LRFS ($P < 0.05$). These genes were used to train a multivariate Cox model with L1 regularization by the least absolute shrinkage and selection operator (LASSO; ref. 26) to obtain the RSS signature (Data Supplementary).

To train the IMS, we started with the antigen presentation and processing (APP) gene set curated by the Immunology Database and Analysis Portal (ImmPort: http://www.immport.org). First, univariate Cox regression analyses were used to identify individual genes that had significant interaction with radiotherapy for predicting DSS. Then a multivariate ridge-regularized Cox model was trained to obtain the IMS signature (Data Supplementary). Because clinical variables such as age, stage, ER/HER2 status, chemotherapy, and hormonal therapy did not significantly interact with radiotherapy, they were not adjusted for when developing the IMS.

Determination of cutoff values for RSS and IMS

We used the maximally selected rank statistics (27) to determine the optimal cutoffs for the RSS or IMS gene signatures to dichotomize patients in the corresponding training cohorts. In particular, for RSS, we computed the standardized log-rank statistics between the two sub-groups dichotomized at different cut points and selected the one that maximized the log-rank statistic. On the other hand, the cutoff value for IMS was selected by dividing patients into two groups in a way that takes into account the interaction effect between the dichotomized gene signature and radiotherapy (as IMS was developed as a predictive signature). Specifically, for any given candidate cutoff point, one group consisted of patients treated with radiation and whose IMS was higher than the candidate cut point, along with patients not treated with radiation and whose IMS was lower than the candidate cut point; the other group consisted of the remaining patients. We then computed the standardized log-rank statistics between the two groups at different candidate cut points for IMS and selected the one that maximized the log-rank statistic. Both signatures and their respective cutoff values were fixed before they were applied to the independent validation cohorts.

Statistical analysis

The log-rank test was used to assess the survival differences between risk groups stratified by gene signatures at the optimal cutoffs or by the treatment status. The *P* value for the interaction term between dichotomized gene signatures and treatment indicator in a multivariate Cox regression analysis was used to assess the predictive significance of gene signatures for radiotherapy benefit. All tests were two sided. A *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using the R software version 3.4.0.

Results

Radiosensitivity signature and its prognostic value

We found 33 radiation-related gene sets from the curated GSEA gene sets. A total of 925 unique genes were available in the microarray platforms used in this study. Among them, 138 genes were significantly correlated with LRFS ($P < 0.05$) on univariate analysis in the training cohort. A 34-gene RSS were obtained on the basis of a LASSO-regularized Cox regression model (Supplementary Table S1). The optimal cutoff value was found to be 1.0 for the RSS in the training cohort (Supplementary Fig. S1). Accordingly, patients with RSS < 1.0 were assigned to the radiation-sensitive group that was associated with better outcomes after radiotherapy, whereas patients with RSS > 1.0 were assigned to the radiation-resistant group that was associated with worse outcomes after radiotherapy.

In the training cohort, RSS was able to stratify patients for LRFS ($P < 0.001$, Supplementary Fig. S2) and was strongly associated with LRFS in both univariate and multivariate analyses (Supplementary Table S2). When tested independently for its prognostic value, RSS was also able to stratify patients for RFS in the two validation cohorts. In particular, patients in the radiation-resistant group had significantly worse RFS compared with those in the radiation-sensitive group in the NKI validation cohort [Fig. 2A, $P = 0.0028$; HR, 1.76; 95% confidence interval (CI), 1.21–2.57] and GSE2034 validation cohort (Fig. 2B, $P = 0.011$; HR, 1.64; 95% CI, 1.12–2.40).

Immune signature and its prognostic value

A total of 119 APP-related genes were available in microarray platforms used in this study. Four genes showed significant interaction with radiotherapy ($P < 0.05$) on univariate analysis in the training cohort. Using ridge-regularized Cox regression, we constructed an immune signature: IMS = 4.7^{*}*ADRM1* + 3.6^{*}*MICB* + 4.8^{*}*PSMD13* – 3.7^{*}*RFXANK*. The optimal cutoff value for the IMS was found to be −3.8 in the training cohort (Supplementary Fig. S3). Accordingly, patients with IMS < −3.8 were assigned to the immune-effective group that was associated with improved survival with radiotherapy, whereas patients with IMS > −3.8 were assigned to the immune-defective group that was associated with worse survival with radiotherapy (Supplementary Fig. S4). IMS showed a strong interaction with radiotherapy in the training cohort in both univariate and multivariate analyses (Supplementary Table S3).

Although IMS was intrinsically developed as a predictive biomarker, we also evaluated its prognostic value on the two prognostic validation cohorts. On the NKI cohort, IMS was able to stratify patients into two groups with distinct RFS, where immune-defective patients had significantly worse RFS than the immune-effective patients (Supplementary Fig. S5A, $P = 0.0031$; HR, 1.89;

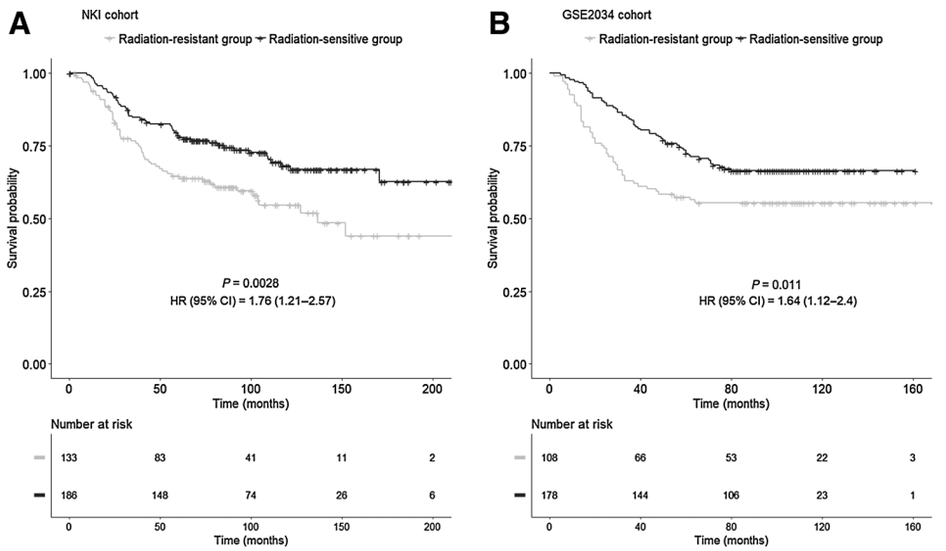

Figure 2.
Relapse-free survival stratified by RSS in the prognostic validation cohorts: (**A**) NKI; (**B**) GSE2034.

95% CI, 1.23–2.91). However, IMS did not stratify the GSE2034 cohort, where both groups had similar RFS (Supplementary Fig. S5B, $P = 0.86$; HR, 0.97; 95% CI, 0.65–1.43).

Predictive value of RSS and IMS

We used the RSS and IMS gene signatures to divide patients in the METABRIC validation cohort into different subgroups. Within each subgroup, we performed exact 1:1 matching between patients treated with and without radiotherapy based on seven clinical and treatment characteristics (Table 1). This matching process obviously reduced the number of patients available in each subgroup. The number of patients excluded and the specific reasons for exclusion were summarized in Supplementary Table S4.

In the METABRIC validation cohort, radiotherapy did not show a survival benefit in terms of DSS either in the entire cohort ($P = 0.60$; HR, 1.05; 95% CI, 0.87–1.27, Supplementary Fig. S6A) or among patients matched for clinical variables ($P = 0.98$; HR, 1.00; 95% CI, 0.76–1.33, Supplementary Fig. S6B). However, in the radiation-sensitive group, patients who received radiotherapy tended to have better DSS than those who did not (Fig. 3A, $P = 0.059$; HR, 0.68; 95% CI, 0.45–1.02). By contrast, a reverse trend was observed in the radiation-resistant group (Fig. 3B, $P = 0.059$; HR, 1.52; 95% CI, 0.98–2.38). There was a significant interaction between RSS and radiotherapy ($P = 0.007$) according to Cox regression analysis, suggesting that RSS could have predictive value.

Similarly, in the immune-effective group, patients treated with radiotherapy had significantly better DSS compared with those without radiation therapy (Fig. 3C, $P = 0.0076$; HR, 0.46; 95% CI, 0.26–0.83). However, there was no significant difference in DSS between the treatment arms in the immune-defective group (Fig. 3D, $P = 0.16$; HR, 1.27; 95% CI, 0.91–1.79). The interaction between IMS and radiotherapy was significant ($P = 0.005$).

To investigate whether integrating both signatures would allow better stratification, we divided patients into three groups. In one concordant group defined by both radiation-sensitive and immune-effective, patients treated with radiation had significantly better DSS than those without radiation (Fig. 4A, $P = 0.022$; HR, 0.43; 95% CI, 0.21–0.90). Conversely, in another concordant group defined by radiation-resistant and immune-defective, patients treated with radiotherapy had significantly worse DSS than those without (Fig. 4B, $P = 0.045$; HR, 1.69; 95% CI, 1.01–2.83). In the discordant group of radiation-sensitive, immune-defective or radiation-resistant, immune-effective patients, there was no significant difference in DSS between the treatment arms (Fig. 4C, $P = 0.36$; HR, 0.81; 95% CI, 0.52–1.27). The interaction between the group labels and radiotherapy was significant ($P = 0.003$).

We performed the same analysis in subgroups of patients as defined by the surgery type. Similar results were obtained for patients who received either mastectomy or BCS, although results for the BCS cohorts were not significant due to a smaller number of patients (Supplementary Figs. S7–S10).

Considering the large number of patients excluded due to exact matching of clinical variables, we also performed multivariate Cox regression analysis of RSS and IMS in the entire METABRIC cohort. The results showed that both RSS and IMS gene signatures had statistically significant interactions with radiotherapy when dichotomized at pre-specified cutoffs (Supplementary Tables S5–S7).

Comparison with previously published gene signatures

We tested two existing gene signatures, the radiation sensitivity index (RSI; ref. 28) and 21-gene Oncotype DX recurrence score (29) for predicting benefit of radiation therapy in the METABRIC cohort. Neither RSI nor Oncotype DX showed a significant interaction with radiotherapy as continuous variables in multivariate Cox regression analyses (Supplementary Tables S8 and S9, $P_{\text{interaction}} = 0.26$, 0.56, respectively). In comparison, RSS showed strong interaction with radiotherapy ($P_{\text{interaction}} = 0.004$, Supplementary Table S5); whereas IMS was borderline significant when assessed as continuous variables ($P_{\text{interaction}} = 0.07$, Supplementary Table S6).

We also evaluated RSI and Oncotype DX as binary variables. For the RSI, we set the cutoff value to be the 25% quantile as suggested by the authors (30). For the Oncotype DX, we used 30 as the cutoff above which patients are assumed to have high risk of recurrence (12, 16). Neither RSI nor Oncotype DX showed significant results for predicting radiotherapy benefit in the METABRIC cohort

Table 1. Clinical and treatment characteristics of the matched METABRIC cohorts

Matching variables[a]	Radiation-sensitive	Radiation-resistant	*P*	Immune-effective	Immune-defective	*P*	Radiation-sensitive, immune-effective	Radiation-resistant, immune-effective or radiation-sensitive, immune-defective	Radiation-resistant, immune-defective	*P*
Total no.	322	202		180	348		126	236	152	
Age ≥ 50 years	280 (87%)	168 (83%)	0.28	148 (82%)	296 (85%)	0.47	102 (80%)	208 (88%)	124 (82%)	0.10
ER positive	282 (88%)	154 (76%)	0.001	176 (98%)	262 (75%)	<0.001	124 (98%)	190 (81%)	110 (72%)	<0.001
HER2 positive	10 (3%)	52 (26%)	<0.001	2 (1%)	56 (16%)	<0.001	2 (2%)	12 (5%)	44 (29%)	<0.001
Stage			<0.001			0.71				0.004
0	2 (1%)	0 (0%)		0 (0%)	2 (<1%)		0 (0%)	2 (<1%)	0 (0%)	
1	86 (27%)	36 (18%)		44 (24%)	82 (24%)		30 (24%)	72 (31%)	24 (16%)	
2	228 (71%)	142 (70%)		126 (70%)	242 (70%)		92 (73%)	152 (64%)	112 (74%)	
3	6 (2%)	22 (11%)		10 (6%)	20 (6%)		4 (4%)	10 (4%)	14 (9%)	
4	0 (0%)	2 (1%)		0 (0%)	2 (<1%)		0 (0%)	0 (0%)	2 (1%)	
Received hormone therapy	258 (80%)	144 (71%)	0.03	150 (83%)	256 (74%)	0.02	102 (81%)	186 (79%)	104 (68%)	0.02
Received chemotherapy	26 (8%)	42 (21%)	<0.001	10 (6%)	58 (17%)	0.001	10 (8%)	18 (8%)	38 (25%)	<0.001
Surgery type			0.02			1				0.10
Mastectomy	242 (75%)	170 (84%)		142 (79%)	276 (79%)		94 (75%)	180 (76%)	128 (84%)	
BCS	80 (25%)	32 (16%)		38 (21%)	72 (21%)		32 (25%)	56 (24%)	24 (16%)	

[a]Due to the exact matching within each of the 7 groups (radiation-sensitive etc.), the matching variables had identical distributions comparing patients who received RT versus those who did not.

(Supplementary Figs. S11 and S12, $P_{interaction} = 0.63$, 0.52, respectively). In multivariate analysis, both RSS and IMS gene signatures had statistically significant interactions with radiotherapy independent of clinical variables and RSI and Oncotype DX scores (Supplementary Table S10).

Discussion

In this study, we developed two gene-expression signatures reflecting distinct biological processes (intrinsic tumor radiosensitivity and immune response) to predict benefit of radiotherapy in breast cancer. We showed in an independent validation cohort that patients with radiation-sensitive or immune-effective tumors derived significant survival benefit from radiotherapy, whereas those with radiation-resistant or immune-defective tumors did not benefit from radiotherapy. Furthermore, we found that integration of radiosensitivity and immune signatures allowed even better stratification of patients in terms of predicting benefit from radiotherapy.

Given the heterogeneity of breast cancer, an individualized approach is needed to guide the optimal use of adjuvant radiotherapy. On the basis of standard clinical and pathological factors, elderly women with small, hormone receptor–positive invasive cancers treated with breast-conserving surgery and endocrine therapy have been the only group identified to date in which omission of radiotherapy can be considered appropriate (31). In the post-mastectomy setting, there have been similar efforts to use combinations of prognostic factors such as tumor size and number of involved nodes to help optimize patient selection for radiation treatment (32, 33). The value of radiotherapy is currently being explored in patients at intermediate risk of recurrence following mastectomy (34).

There has been growing interest in integrating tumor biology information in addition to clinical and pathological factors to improve decision-making and individualization of radiation treatment (35–37). While prognostic biomarkers can identify patients with poor outcomes irrespective of treatment, predictive biomarkers inform the likelihood of response to and potential benefit from a specific therapy, and thus have more direct clinical relevance (38). Intrinsic subtyping by immunohistochemistry has been reported to be prognostic for breast cancer recurrence (39, 40), but has not been shown to predict benefit from radiotherapy (41, 42). In addition, several gene signatures have been proposed to estimate risk of locoregional recurrence after BCS or mastectomy (10–16), but few have been successfully validated in independent cohorts (19). A more fundamental issue is that these gene signatures are prognostic by design because they were developed in patients who all received radiotherapy without using an appropriate control group, that is, patients who did not receive radiotherapy.

Although tumor intrinsic radiosensitivity is widely accepted for its direct impact on radiation response, various factors in the tumor microenvironment also influence and modulate therapeutic effects (17). Radiation can prime the immune system by inducing immunogenic cell death which releases neoantigens (43–46), potentially eliciting systemic antitumor immune response (i.e., abscopal effect; ref. 47). A critical step during this process is the ability of the immune system to recognize cancer cells by tumor-associated antigens presented on their MHC molecules (18). Accordingly, cancer cells with normal APP function are more likely to be recognized and killed by cytotoxic T cells

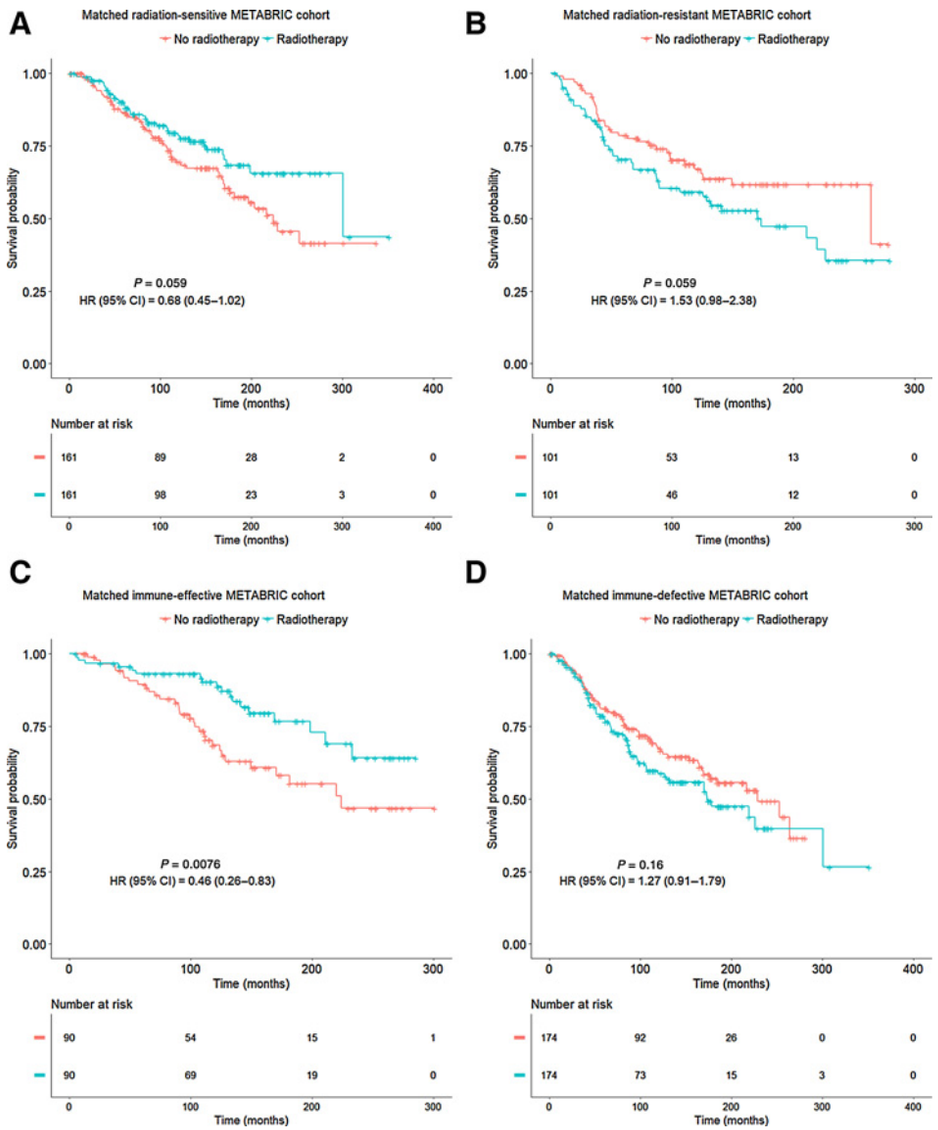

Figure 3.
DSS stratified by RSS in the METABRIC cohort: (**A**) matched radiation-sensitive cohort; (**B**) matched radiation-resistant cohort; $P_{interaction} = 0.007$. DSS stratified by IMS: (**C**) matched immune-effective cohort; (**D**) matched immune-defective cohort; $P_{interaction} = 0.005$.

than those with dysregulated APP function. In the setting of radiotherapy, such differences in APP function may influence response and outcomes through the contribution of radiation-induced immune priming (43). Indeed, our immune signature which was derived solely from APP-related genes could distinguish between patients who benefitted from radiotherapy and those who did not. Interestingly, mutations in the gene encoding β-2-microglobulin, which is necessary for the folding and transport of MHC class I molecules to the cell surface for antigen presentation, have recently been shown to promote resistance to anti-programmed cell death 1 (PD1) immunotherapy (48). To be clear, other factors such as immune checkpoints (49) or immunosuppressive cytokines (50) may also play an important role in mediating tumor and host response to radiation, and should be explored in future studies (51).

The proposed RSS and IMS gene signatures were related to but not identical to established clinicopathologic variables. In our analyses, there was a higher proportion of ER-positive patients within the radiation-sensitive and immune-effective groups compared with others in the matched METABRIC validation cohort (Table 1). This result is consistent with the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis (1), which showed that among all groups defined by traditional clinicopathologic factors, ER-positive tumors had the greatest response to radiotherapy (RT). However, not all ER-positive tumors would be expected to benefit from RT. Accordingly, approximately 1 out of 3, and 3 out of 5 ER-positive tumors were classified as radiation-resistant and immune-defective by gene expression, respectively. Our proposed signatures might help select ER-positive patients who are most likely to benefit from RT. On the other hand, the potentially higher-risk HER2-positive tumors were significantly enriched in the radiation-resistant and immune-defective groups in the matched METABRIC cohort. This result is consistent with a recent study (42) showing that HER2-positive tumors are most radioresistant among all subtypes and thus may not benefit from RT.

The main limitation of our study is the use of retrospective cohorts with nonrandomized treatment with radiotherapy.

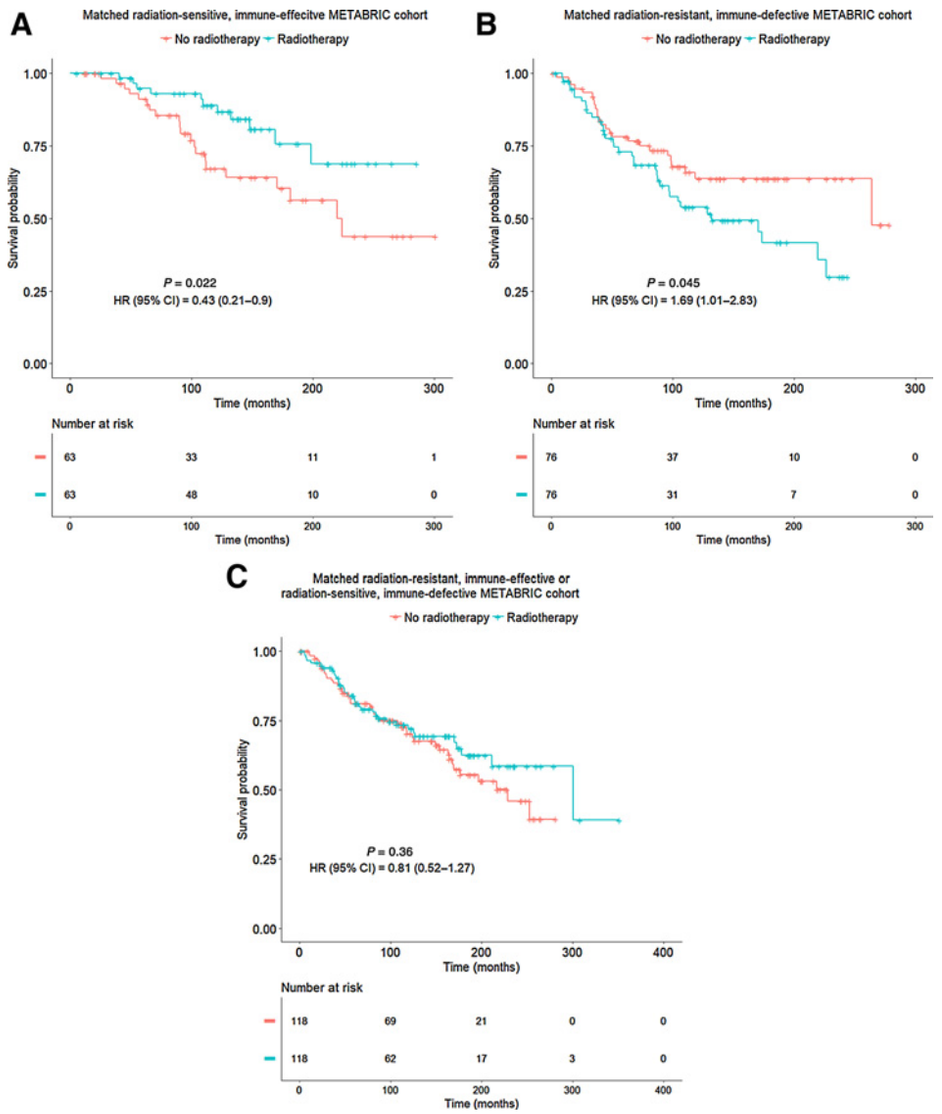

Figure 4.
DSS stratified by integrating RSS and IMS in the METABRIC cohort. **A,** Matched radiation-sensitive, immune-effective cohort; (**B**) matched radiation–resistant, immune-defective cohort; (**C**) matched radiation-resistant, immune-effective or radiation-sensitive, immune-defective cohort. $P_{interaction} = 0.003$.

Predictive biomarkers should ideally be tested in prospective randomized controlled trials. To mitigate the potential selection bias in retrospective cohorts, we used a matched strategy to balance relevant clinical and treatment characteristics between comparison groups. A recent study used a similar strategy to develop a radiation-related gene signature for predicting radiotherapy benefit in prostate cancer (52). One key difference is that although Zhao and colleagues (52) matched patients in the entire cohort, we matched patients within each biomarker-defined group (e.g., radiation-sensitive), which ensures balanced cohorts and allows for more rigorous validation.

As previously curated public datasets were used in our study, specific information about locoregional recurrence was not available in some of the validation cohorts, and consequently different endpoints (relapse-free survival or disease-specific survival) were used in some of the validation cohorts. This precludes a direct comparison between different cohorts and could hamper interpretation of the results. On the other hand, although locoregional recurrence is the most direct endpoint for measuring the benefit of RT, survival-related endpoints used in our validation are also clinically relevant because improving survival is the ultimate goal of adjuvant treatment, including RT. Another limitation due to the use of public data is that information on radiation fields was not available so the impact of breast versus nodal irradiation could not be assessed.

In terms of surgery, a majority (~80%) of patients in the METABRIC validation cohort received mastectomy, and fewer (~20%) patients received BCS. Although statistical significance was not reached for the BCS group due to low numbers, we found similar trend in this surgical subgroup as our main results. Future validation is warranted to confirm the predictive value of our gene signatures in the context of BCS. However, as these gene signatures are reflective of the biology of breast cancer, it is likely that they may be equally predictive in both the post-mastectomy and post-lumpectomy settings.

In conclusion, we developed radiosensitivity and immune gene signatures for predicting benefit of radiotherapy in breast cancer

and validated them in a matched retrospective cohort. Should these results be confirmed in future prospective randomized trials, the gene signatures may be used to select patients for whom de-escalation for treatment maybe an option, or to identify patients who may not respond well to standard radiotherapy and may therefore benefit from the addition of radiosensitizers or immunotherapy to enhance the radiation response.

Disclosure of Potential Conflicts of Interest

E.L. Pollom is a consultant/advisory board member for Novocure. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: Y. Cui, B. Li, E.L. Pollom, R. Li
Development of methodology: Y. Cui, B. Li, E.L. Pollom
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Cui, R. Li
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Cui, B. Li, E.L. Pollom, K.C. Horst, R. Li
Writing, review, and/or revision of the manuscript: Y. Cui, B. Li, E.L. Pollom, K.C. Horst, R. Li
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Cui, R. Li
Study supervision: Y. Cui, R. Li

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