High PDGFRb Expression Predicts Resistance to Radiotherapy in DCIS within the SweDCIS Randomized Trial

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ABSTRACT

Purpose: This study analyzes the potential of stromal platelet-derived growth factor receptor-beta (PDGFRb) expression as biomarker for radiotherapy (RT) benefit on ipsilateral breast events (IBE) in ductal carcinoma *in situ* (DCIS). Improved identification of DCIS patients refractory to adjuvant whole-breast RT is needed. Predictive biomarker studies in DCIS have focused on tumor cell features rather than the tumor-associated stroma, despite growing evidence of its influence on therapy efficiency.

Experimental Design: Samples from the Swedish randomized radiotherapy DCIS trial (SweDCIS) were subjected to IHC analysis for stromal PDGFRb expression. IBE incidence at 10 years after breast-conserving surgery was the primary endpoint. Interactions between marker and treatment were analyzed.

Results: PDGFRb score was predictive for RT benefit with regard to IBE ($P_{interaction} = 0.002$ and $P_{interaction} = 0.008$ adjusted multivariably). Patients of the PDGFRb^{low} group had a strong benefit from RT regarding IBE risk [HR, 0.23; 95% confidence interval (CI), 0.12–0.45; $P < 0.001$] with an absolute risk reduction of 21% (cumulative risk 7% vs. 28%) at 10 years. No significant risk reduction by RT was observed for patients of the PDGFRb^{high} group (HR, 0.83; 0.51–1.34; $P = 0.444$; cumulative risk 22% vs. 25%). The RT response–predictive effect of stromal PDGFRb was equally strong in analyses for *in situ* and invasive IBE when analyzed separately (*in situ* IBE: $P = 0.029$; invasive IBE: $P = 0.044$).

Conclusions: Results suggest high stromal PDGFRb expression as a novel biomarker identifying DCIS patients who are refractory to standard whole-breast adjuvant RT. The data imply previously unrecognized fibroblast-mediated modulation of radiosensitivity of DCIS, which should be further explored from mechanistic and targeting perspectives.

Introduction

Ductal carcinoma *in situ* (DCIS) is a preinvasive form of breast cancer with overall good prognosis (1, 2). DCIS accounts for about 10% of all diagnosed breast cancers in Sweden (3) and up to 25% within screened populations in Europe and the USA (4–6). Early studies indicated that only one third of the DCIS patients treated with breast-conserving surgery (BCS) would develop local recurrences, whereas studies including patients diagnosed within the past three decades indicate that recurrence rates after BCS have declined to around 20% at 10 years, most likely due to improvements in detection and pathologic assessment (7, 8).

Breast-conserving therapy, comprising BCS followed by adjuvant radiotherapy (RT), represents the DCIS standard of care based on four prospective randomized studies (9–13). All studies demonstrated a relative risk reduction of ipsilateral breast events (IBE) by approximately 50% at 10 years. However, the present recommendations for RT include a risk of overtreatment or ineffective treatment. Traditional clinicopathologic characteristics and epithelial molecular markers have been assessed for their prognostic utility for IBE risk (14, 15). Still, there remains no standardized or universally accepted pathologic definition of "low risk" as it pertains to the omission of RT for DCIS patients (16).

Investigated markers or signatures have mostly been selected based on their associations with recurrence risk, but there is a need to identify clinical, histology-based or molecular markers predicting direct RT benefit (17–20). A meta-analysis of the four randomized clinical trials concluded that RT was effective regardless of clinicopathologic parameters such as age, detection mode, tumor margins, nuclear grade, or tamoxifen treatment (21).

Growing evidence implies a role of the tumor microenvironment in DCIS progression and treatment response (19, 22–25). Various tumor-supportive or tumor-restraining properties have been linked to stroma cells (reviewed in refs. 26, 27) and associated with disease outcome (refs. 28–30; reviewed in ref. 31). Also, cancer cell–activated fibroblasts exhibited radiation-protective effects on tumor cells in experimental settings (25, 32, 33; reviewed in ref. 34).

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Translational Relevance

Adjuvant radiotherapy after breast-conserving surgery is currently the common treatment modality to reduce ipsilateral breast recurrences for ductal carcinoma *in situ* (DCIS) patients. However, around 10% of patients still experience an ipsilateral breast recurrence within the first decade of diagnosis. There is urgent need for predictive biomarkers to aid in the individualization of radiotherapy treatment for DCIS. Predictive biomarker studies in DCIS have so far focused on tumor cell features rather than on the tumor-associated stroma, despite growing evidence of its influence on therapy efficiency. Cancer-associated fibroblasts are a heterogeneous group of stroma cells that have been implicated in tumor radioresistance, and platelet-derived growth factor receptor-beta (PDGFRb) is a major receptor involved in fibroblast regulation and activation. The presented study suggests high stromal PDGFRb expression as a novel biomarker identifying DCIS patients who are refractory to standard whole-breast adjuvant radiotherapy and thus represent a patient group that might benefit from intensified treatment.

The platelet-derived growth factor (PDGF) signaling pathway is a major regulator of stroma cells (31). In experimental models, platelet-derived growth factor receptor-beta (PDGFRb) signaling in fibroblasts supported tumor cell invasiveness (29), cancer stem cell properties (35), and increased the interstitial fluid pressure (28). In agreement, a high expression of stromal PDGFRb was associated with poor prognosis in many cancer types (23, 36–39). For DCIS, studies have indicated a loss of PDGFRa in the tumor-associated stroma, while PDGFRb remained high, and patients with $PDGFRa^{low}/PDGFRb^{high}$ expression displayed a significantly increased risk for disease recurrence (23). Whether this outcome association reflected effects of the marker on natural course or treatment benefit was not resolved.

The main purpose of the presented study was to analyze whether stromal PDGFRb expression is associated with RT effect in women with DCIS. Therefore, PDGFRb protein expression was evaluated in a large clinical trial cohort randomized for standard whole-breast adjuvant RT after BCS with long-term clinical follow-up (10, 11).

Materials and Methods

Patient cohort

The study population was based on the national Swedish randomized DCIS trial (SweDCIS), in which 1,046 women diagnosed between 1987 and 1999 underwent BCS for primary DCIS and were randomly assigned to a postoperative RT or control arm (10, 11). Microscopically tumor-clear margins were achieved in >80% of cases. Only about 3% of all women received tamoxifen. Clinical trial results and 20-year follow-up were published (10, 11). Reported endpoints include IBE (*in situ* or invasive), contralateral breast events (CBE), distant recurrence, breast cancer–specific death (BCSD), or other deaths. The SweDCIS study and companion analyses were approved by the institutional ethics committee of Umeå University (Fek1987-05-05§2, Dnr05:065M, Dnr2012-224-32M, Dnr2014-230-32M); no written informed consent was needed. The study was further performed in accordance with the Declaration of Helsinki. Of the originally 1,046 patients, tissue blocks for biomarker studies were accessible from 897 patients. Hematoxylin/eosin-stained sections were reevaluated by an independent pathologist. Within 181 cases, no DCIS lesion could be detected, and those cases were excluded from the analysis (**Fig. 1**). Sections of the remaining 716 patients were subjected to PDGFRb IHC. The staining was successful for 590 patients, who were included in the final study cohort, with a median follow-up of 17.4 years.

IHC and marker evaluation

IHC for PDGFRb as predefined single marker was performed largely as described earlier (see Supporting Information for details on marker selection, other markers analyzed in the SweDCIS material, and staining protocol; ref. 23). PDGFRb-stained slides were scanned at 20× magnification on the Hamamatsu Nanozoomer S60 (Hamamatsu). Scoring was performed by two independent raters (C. Strell and D. Folkvaljon) under guidance of an experienced breast pathologist (L.A. Akslen) blinded to clinical and outcome information (see Supplementary Fig. S1 and Supporting Information for details). The PDGFRb scoring data were split at the median and referred to as $PDGFRb^{low}$ and $PDGFRb^{high}$ group (Supplementary Fig. S1C). Interaction analysis was also performed with the continuous score, as recommended (40).

Two additional scoring approaches were also used, which are explained in detail in the Supporting Information as is the assessment of other clinicopathologic characteristics.

Statistical analysis

The predefined primary endpoint of this exploratory retrospective study was IBE incidence at 10 years after BCS, while treating CBE, distant recurrence, BCSD, and death of any cause as competing risks. Secondary endpoints were CBE, distant recurrence, BCSD, and death of any cause within 20 years after BCS.

R-Studio Version1.0.143 was used for statistical analyses. Cumulative incidence functions in the presence of competing risks were generated by the cmprsk package including Gray's test for differences. Patients at risk tables were added using the cr17 package. Hazard ratios (HR) for IBE at 10 years post BCS as the primary endpoint were calculated by Cox proportional hazards regression with the Efron method for ties (survival package), treating CBE as censoring (as potential differences in treatment/monitoring of patients with CBE might affect subsequent IBE). Reported *P* values are based on Wald test. For all secondary endpoints, HRs at 20 years were calculated by cause-specific Cox proportional hazards regression. Multivariable analyses including an interaction term between the PDGFRb score and RT with or without adjustment for indicated covariates were performed to evaluate the RT effect in dependency of PDGFRb expression. For Cox regression analysis, the assumption of proportional hazards was analyzed graphically through evaluation of parallelism of the $\log(-\log(S(t)))$ versus time plot as well as through the Schoenfeld residuals test. A violation of the proportional hazards assumption ($P < 0.05$) was noted for RT effects on IBE; thus, these values should be interpreted as the mean value over 10 years. Time window–based analyses (0–5, 5–10, and 10–20 years) were also conducted (**Table 2** and Supplementary Table S5). Marginal effect of the interaction term between the continuous PDGFRb score and RT was determined by fitting a cause-specific Cox proportional hazards regression model over the first ten years after BCS and application of the plot_model function of the sjPlots package to compute predicted IBE risk scores (model in Supplementary Data).

Group distributions of clinicopathologic parameters were analyzed by contingency tables and Fisher exact test. Ninety-five percent confidence intervals (CI) are given and all statistical tests were two-sided. *P* values <0.05 were considered statistically significant.

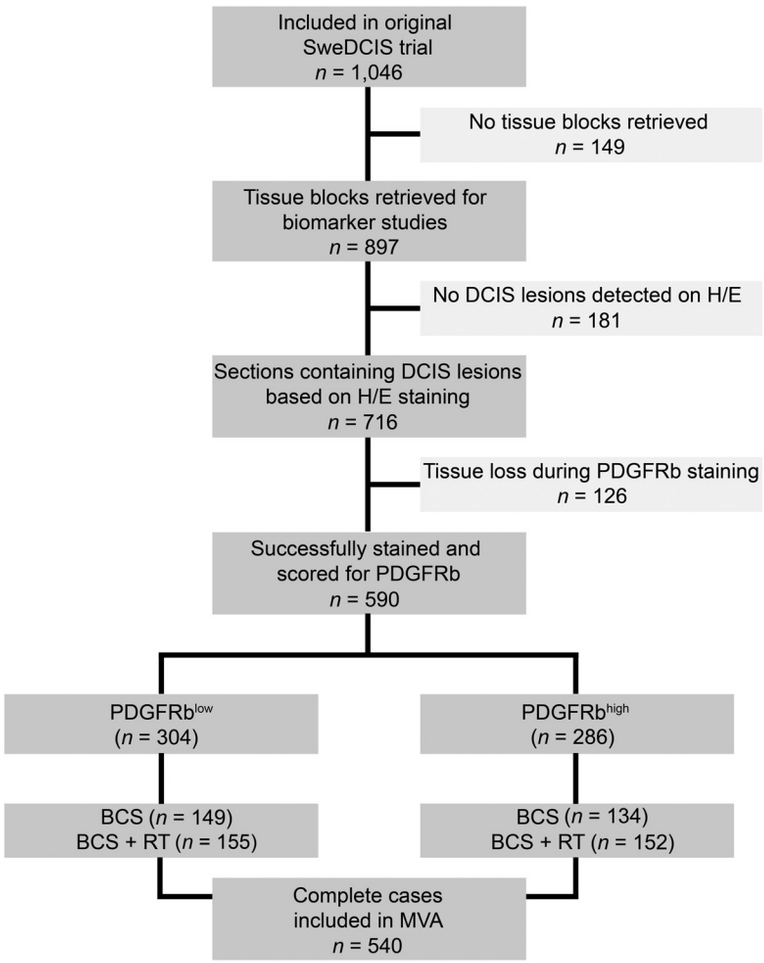

Figure 1.
CONSORT flowchart. Patients from the Swedish DCIS-randomized radiotherapy trial (SweDCIS) included in the present biomarker study. BCS, breast-conserving surgery; MVA, multivariable analysis; RT, radiotherapy.

Results

Patient characteristics

Tissue sections containing DCIS lesions were available for 716 patients of the 1,046 women in the SweDCIS trial. Of these, 590 samples were successfully stained for PDGFRb (corresponding to a loss of 17.6% during staining procedure) and were included in the presented biomarker study (**Fig. 1**).

Clinicopathologic patient characteristics and event rates for those 590 patients did not differ from the whole SweDCIS cohort (Supplementary Table S1). The study cohort retained the IBE risk reduction by RT as described for the SweDCIS cohort (ref. 10; Supplementary Fig. S2). The control and RT arms of the study cohort remained well balanced regarding clinical characteristics (Supplementary Table S2).

For each rater, the average PDGFRb score per patient was calculated (see Materials and Methods and Supplementary Fig. S1). The Cohen kappa coefficient (κ) of 0.712 indicated a substantial degree of agreement (Supplementary Fig. S1B; ref. 41). For all subsequent analyses, the overall average of the two raters was calculated. Patients were dichotomized into PDGFRb "low" ($n = 304$; 51.5%) and "high" ($n = 286$; 48.5%) groups using the median score (1.65) as cutoff (Supplementary Fig. S1C).

No association between PDGFRb status and clinicopathologic characteristics including nuclear grade, Ki67 index, and infiltrating lymphocytes was noted, except for a tendency toward younger age in the PDGFRb^{high} group (Supplementary Table S3). Clinicopathologic characteristics remained well balanced within control and RT treatment arms of each PDGFRb-defined subgroups (Supplementary Table S2).

Prognostic value of stromal PDGFRb

In order to investigate the impact of stromal PDGFRb expression on the natural course of the disease post BCS, analyses were performed for the control no-RT patients regarding associations between marker and outcome-related endpoints. There was no statistically significant difference for IBE incidence as first event within 10 years between the PDGFRb groups (**Fig. 2**, left graph). Likewise, no prognostic impact for PDGFRb expression was observed for the secondary endpoints CBE, distant recurrence, BCSD, and overall survival among the no-RT patients (Supplementary Table S4).

Analyses of the RT-treated patients indicated a statistically significant higher IBE risk for the PDGFRb^{high} group as compared with those of the PDGFRb^{low} group (HR, 3.26; 95% CI, 1.65–6.45; $P < 0.001$ Wald test; **Fig. 2**, right graph), while no prognostic

Table 1. Interaction of PDGFRb score and radiotherapy with regard to IBE at 10 years after BCS.

	HR (95% CI)	*P* value
UVA (total $n = 590$)		
PDGFRb score (low/high)	1.38 (0.98–1.99)	0.082
Radiotherapy (no/yes)	0.50 (0.35–0.73)	**<0.001**
Interaction (total $n = 590$)		
PDGFRb score (low/high)	0.89 (0.57–1.41)	0.630
Radiotherapy (no/yes)	0.23 (0.12–0.45)	**<0.001**
PDGFRb score: radiotherapy	3.59 (1.58–8.18)	**0.002**
Interaction MVA adjusted (total $n = 540$)		
PDGFRb score (low/high)	0.93 (0.57–1.53)	0.782
Radiotherapy (no/yes)	0.26 (0.13–0.51)	**<0.001**
Age (<50 years reference)	1	—
50–60 years	1.18 (0.71–1.95)	0.523
>60 years	0.96 (0.56–1.65)	0.893
Size[a] (≤15 mm/>15 mm)	2.40 (1.61–3.57)	**<0.001**
Nuclear grade (1 as reference)	1	—
2	1.51 (0.88–2.59)	0.131
3	1.47 (0.82–2.66)	0.197
Ki67[b] (<20%; ≥20%)	1.17 (0.74–1.84)	0.505
Endocrine therapy (no/yes)	0.89 (0.28–2.81)	0.839
Screen detected[c] (screen/clinical)	1.51 (0.93–2.44)	0.096
PDGFRb score: radiotherapy	3.21 (1.36–7.57)	**0.008**

Note: *P* values are based on Wald test. Significant *P* values are marked in bold.
Abbreviations: BCS, breast-conserving surgery; CI, confidence interval; IBE, ipsilateral breast event; MVA, multivariable analysis.
[a]Data missing $n = 43$.
[b]Data missing of four patients.
[c]Data missing of three patients.

impact for PDGFRb expression was observed for the secondary endpoints (Supplementary Table S4).

Effect of RT on IBE as first event in PDGFRb-defined patient groups

To further investigate the impact of stromal PDGFRb expression on postoperative RT benefit, IBE incidences were analyzed within the PDGFRb-defined groups. For the PDGFRb^{low} group, a reduced cumulative incidence for IBE (*in situ* and invasive) as first event within 10 years after BCS was observed for the RT arm compared with control arm (0.07; 95% CI, 0.04–0.12 vs. 0.28; 0.21–0.35), corresponding to an absolute risk reduction of 21% by RT at 10 years after BCS ($P < 0.001$, Gray test; **Fig. 3A**). In this group, the RT-treated patients had one fifth of the risk for IBE as patients not treated with RT (HR 0.23; 95% CI, 0.12–0.45; $P < 0.001$ Wald test). In contrast, within the PDGFRb^{high} patient group, no significant difference was detected between the RT and control arms (cumulative incidence for IBE at 10 years, 0.22; 0.16–0.29 vs. 0.25, 0.18–0.32; HR, 0.83; 0.51–1.34; $P = 0.444$ Wald test; **Fig. 3A**).

The predictive capacity of the marker for RT benefit was demonstrated in a formal interaction test ($P_{interaction} = 0.002$; **Table 1**), which remained significant after adjustment for age, size, nuclear grade, Ki67 index, screening detection, and endocrine treatment ($P_{interaction} = 0.008$; **Table 1**). A statistically significant interaction was also noted in analyses using the continuous PDGFRb score ($P_{interaction} = 0.009$ before and 0.040 after multivariable adjustment; **Fig. 3B**).

Similar results were obtained in analyses using data from the two alternative scoring methods; the "hot spot"- or the "overall integrated"-scoring approaches (see Supporting Materials and Methods and Supplementary Fig. S3).

Effect of RT on *in situ* and invasive IBE separately

About 60% of all IBE were *in situ*, whereas 40% were of an invasive IBE phenotype, at 10 years after BCS. Importantly, analyses of *in situ* and invasive IBE separately indicated that for each recurrence type, the RT effect was more pronounced in the PDGFRb^{low} patient group as compared with the PDGFRb^{high} group (Supplementary Table S2; **Fig. 4**). Among the patients of the PDGFRb^{low} group, a statistically significant relative risk reduction by RT was observed for both IBE types within 10 years after BCS (*in situ*: HR, 0.22; 0.10–0.50; $P < 0.001$ Wald test; invasive: HR, 0.26; 0.08–0.79; $P = 0.017$), while among the PDGFRb^{high} patients, no risk reduction was observed (*in situ*: HR, 0.70; 0.37–1.34; $P = 0.283$; invasive: HR, 1.02; 0.49–2.13; $P = 0.949$). The interaction of PDGFRb score and RT was significant for *in situ* IBE ($P_{interaction} = 0.029$ and 0.048 after multivariable adjustment for age, size, nuclear grade, Ki67 index, screening detection, and endocrine treatment) as well as invasive IBE ($P_{interaction} = 0.044$ and 0.101 after multivariable adjustment; **Fig. 4**).

Effect of RT on IBE for PDGFRb-defined patient groups across time-stratified subgroups

In order to analyze possible time dependency of the PDGFRb association with treatment benefit, analyses were done for IBE rates in PDGFRb- and RT-defined populations during 0–5-, 5–10-, and 10–20-year spans after BCS.

The annual average IBE rate for the PDGFRb^{low} patient group treated with RT was 0.8% during 0–5 years and 0.6% during 5–10 years, whereas the untreated control patients showed annual IBE rates of 4.0% and 1.5%, respectively, during these time periods (**Table 2**). Of

Table 2. Average cumulative incidence rate per year of IBE (*in situ* and invasive) as percentage within PDGFRb-defined patient groups over time after BCS in comparison with CBE as baseline.

	IBE						
	PDGFRb^{low}			PDGFRb^{high}			
Time (years)	No RT	RT	*Difference (no RT – RT)*	No RT	RT	*Difference (no RT – RT)*	CBE all
0–5	4.0%	0.8%	*3.2%*	3.9%	2.1%	*1.8%*	0.5%
5–10	1.5%	0.6%	*0.9%*	1.0%	2.2%	*−1.2%*	0.5%
10–20	0.6%	0.5%	*0.1%*	0.4%	0.3%	*0.1%*	0.4%
0–20	1.7%	0.6%	*1.1%*	1.4%	1.2%	*0.2%*	0.5%

Abbreviations: BSC, breast-conserving surgery; CBE, contralateral breast event; IBE, ipsilateral breast event; RT, radiotherapy.

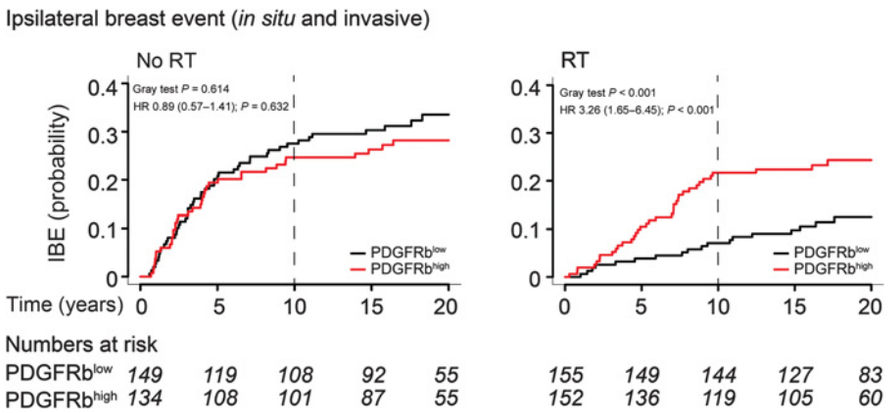

Figure 2.
Prognostic performance of stromal PDGFRb expression within RT-stratified patient groups. Graphs show cumulative incidence of IBE (*in situ* and invasive) since BCS. Graphs are derived from competing risk analyses with IBE as first events treating CBE, distant recurrences, BCSD, and any death of any cause as competing risks. The Gray test was applied for comparison of the cumulative incidence functions at 10 years after BCS. HRs (PDGFRb^{low} group as reference) at 10 years are indicated with 95% CI (in parenthesis) and *P* values are based on the Wald test. IBE, ipsilateral breast event; RT, radiotherapy.

note, the IBE rates of the RT-treated patients were thereby similar to the average annual CBE rate, which serves as a candidate proxy for new primary tumor risk (0.5% between 0–5 and 5–10 years, respectively). In contrast, RT-treated PDGFRb^{high} patients had greater incidence rates than the annual CBE rate during the 0–5- as well as 5–10-year time period (2.1% and 2.2% per year, respectively; **Table 2**). The predictive capacity of the PDGFRb score for RT benefit was demonstrated in a formal interaction test both between 0–5 years ($P_{interaction} = 0.025$) and 5–10 years ($P_{interaction} = 0.006$; Supplementary Table S5).

From 10 years after BCS and onward, the average IBE rate was not different between any of the PDGFRb-defined groups with or without RT, and resembled the annual risk rate of CBE (**Table 2**).

Effect of RT on CBE, distant recurrence, BCSD, and overall survival in PDGFRb-defined patient groups

The impact of stromal PDGFRb expression on the effect of RT on secondary endpoints was investigated. No statistically significant effect of RT was observed for any of the secondary endpoints in the PDGFRb-defined patient groups within 20 years after BCS (Supplementary Table S6).

Discussion

The presented retrospective biomarker study indicates that high stromal PDGFRb expression is a previously unrecognized candidate

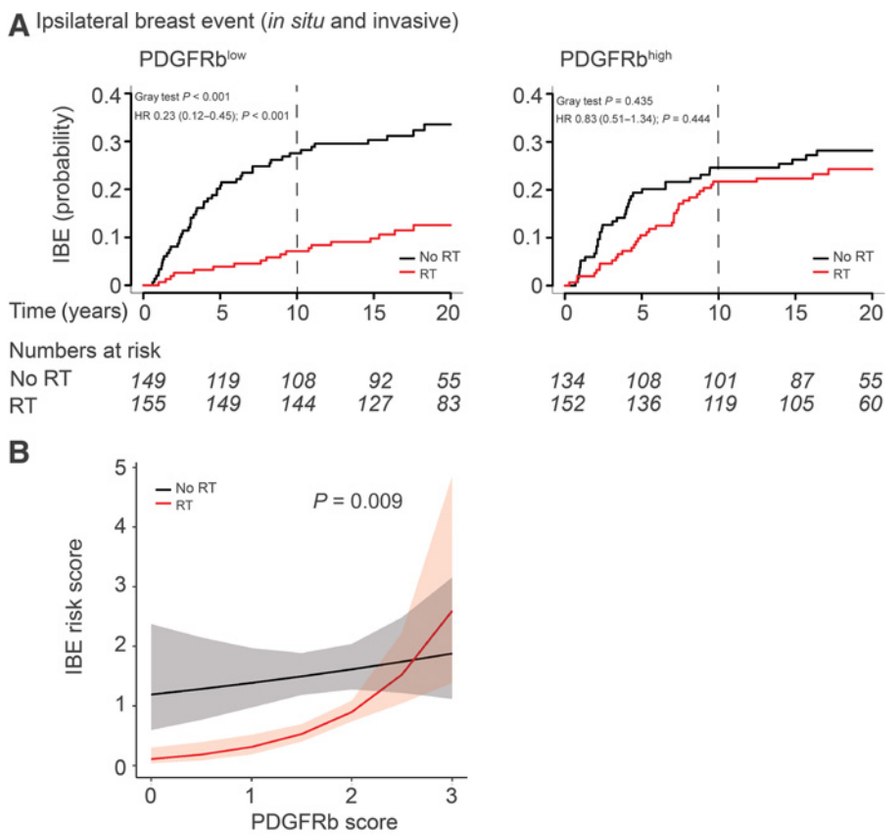

Figure 3.
Predictive performance of stromal PDGFRb expression for the benefit of radiotherapy on IBE in DCIS. **A,** Effect of standard whole-breast adjuvant radiotherapy in PDGFRb-defined "low" and "high" subsets (as split by median score). Graphs show cumulative incidence of IBE (*in situ* and invasive) since BCS. Graphs are derived from competing risk analyses with IBE as first events treating CBE, distant recurrences, BCSD, and any death as competing risks. The Gray test was applied for comparison of the cumulative incidence functions at 10 years after BCS. HRs (no-RT group as reference) at 10 years are indicated with 95% CI (in parenthesis), and *P* values are based on the Wald test. **B,** Margin plot of the interaction between the continuous PDGFRb score and RT treatment with predicted risk scores for IBE (*in situ* and invasive) at 10 years. The average 10-year IBE risk score was calculated by fitting a Cox regression model to time to IBE using the interaction term of the continuous PDGFRb score and RT treatment. CI, confidence interval; IBE, ipsilateral breast event; RT, radiotherapy.

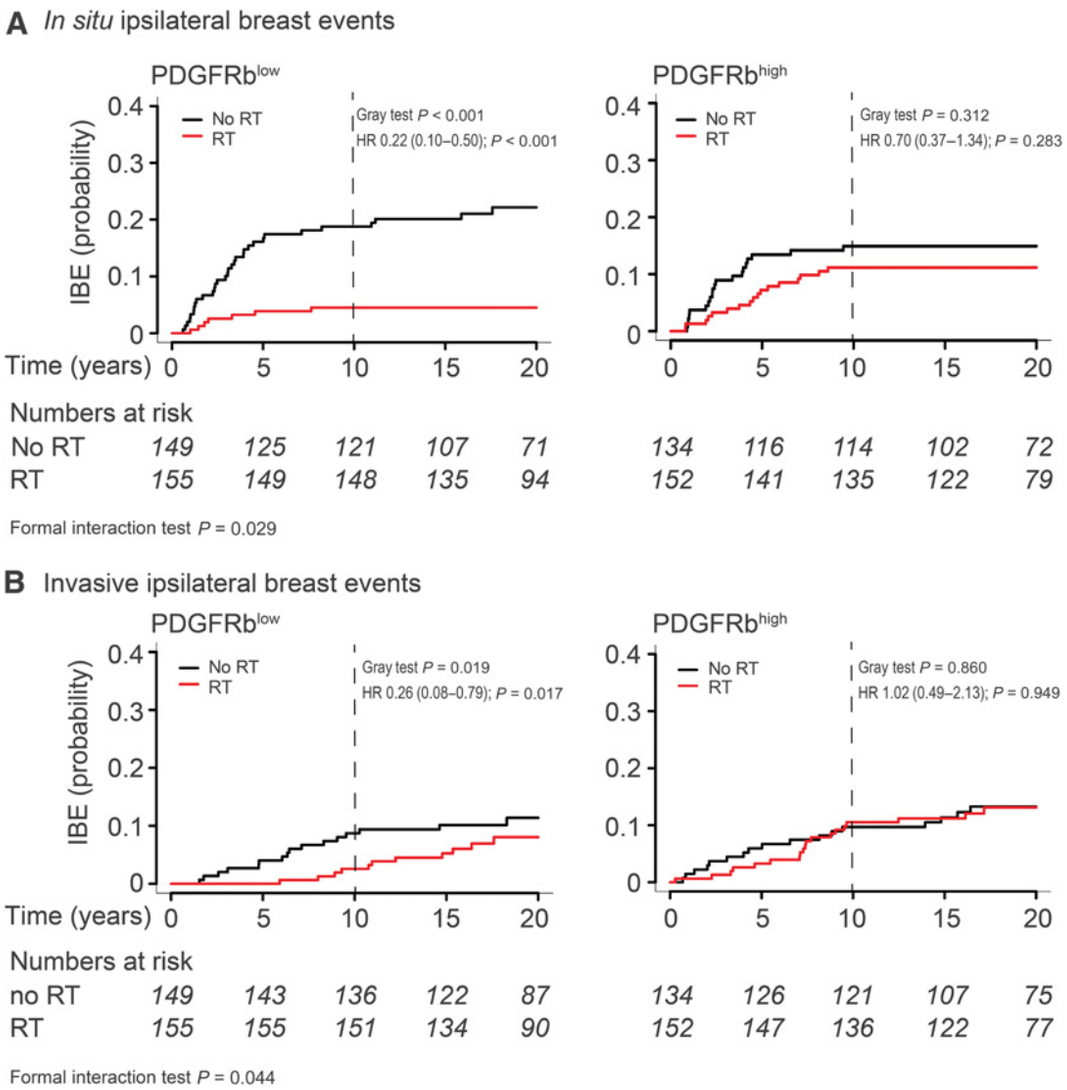

Figure 4.

RT effect in PDGFRb-defined patient subgroups separate for *in situ* (**A**) and invasive (**B**) IBE. Graphs show cumulative incidence of (**A**) *in situ* IBE and (**B**) invasive IBE in dependency of radiotherapy in PDGFRb-defined patient subgroups. The Gray test was applied for comparison of the cumulative incidence functions over 10 years after BCS. HRs (no-RT group as reference) are indicated with 95% CI (in parenthesis) and *P* values are based on the Wald test. Ninety-three percent of the *in situ* IBE were detected during the first ten years since BCS, as thereafter most of the women were no longer included in national screening programs based on their age. RT, radiotherapy.

marker to identify DCIS patients refractory to adjuvant RT regarding the IBE risk within 10 years after BCS. This interaction between RT and PDGFRb status was confirmed in a formal interaction test including marker and treatment only, as well as in multivariable analysis adjusted for age, size, Ki67 index, nuclear grade, screening detection, and endocrine treatment (**Table 1**). Importantly, among the patients with low PDGFRb expression, the RT benefit was equally noted with regard to *in situ* as well as invasive IBE. The reduced RT benefit in PDGFRb^{high} patients was pronounced between 5 and 10 years after BCS and likely caused by a delayed clinical detection of recurrences as a consequence of post-radiation changes within the breast tissue (42).

Taking the annual incidence rate of CBE (0.5%) as a measure of new primary risk, one may conclude that PDGFRb^{low} DCIS patients receiving BCS and RT have a cumulative IBE incidence rate approaching CBE risk. No prognostic effect of PDGFRb expression on the natural course of disease after BCS was detected in RT-naïve patients.

To the best of our knowledge, this represents the first study identifying a statistically significant interaction between a stromal marker and benefit of RT for DCIS in large randomized trial. Of note, the used study material was collected as part of the SweDCIS trial, one of the four studies driving the present recommendation for RT following BCS. In spite of these strengths, the exploratory nature of this study is recognized. Continued analyses, including validations in independent cohorts, are thus important tasks for future studies.

Future studies should also aim to decipher the functional mechanisms underlying the association between high stromal PDGFRb

expression and reduced RT sensitivity. Different stroma cell–derived factors affect tumor biology through paracrine signaling networks in a manner potentially relevant for radiation sensitivity (reviewed in ref. 34). Previous preclinical mechanistic studies have demonstrated that stromal PDGFRb signaling can support tumor growth, negatively regulate tumor drug uptake, and enhance metastasis (ref. 28–30; reviewed in ref. 31). Regarding DCIS, it was recently shown that stromal PDGFR status was associated with outcome in a manner where $PDGFRa^{low}/PDGFRb^{high}$ was associated with poor prognosis (23). In the same study, this fibroblast phenotype was found to be associated with increased signaling activity and ligand production of TGF-beta, a considered modulator of tumoral radiosensitivity (34). It will be an important task to perform experimental studies investigating how specifically PDGFRb-signaling manipulations will affect sensitivity in *in vivo* models of adjuvant RT. Notably, findings from such studies will eventually suggest novel radiosensitizing combination therapies.

It is recognized that the presented results are also compatible with a model of PDGFRb being solely a marker, rather than driver of a fibroblast-subset affecting RT sensitivity. A series of studies have identified immune-modulatory fibroblasts, most commonly defined by high expression of fibroblast activation protein (FAP; refs. 43, 44). Notably, data from a randomized RT study of stage I/II invasive breast cancer demonstrated that low numbers of tumor-infiltrating lymphocytes were associated with larger RT benefit (45).

Based on the presented findings, follow-up studies applying novel techniques for multiplexed detection of fibroblast and other mesenchymal markers to the SweDCIS cohort are indeed motivated. A prioritized candidate marker in such studies is PDGFRa, based on the earlier findings indicating PDGFRa as a marker for a functionally distinct fibroblast subset (23). Multiplexed marker analyses could potentially provide more precise definitions of cellular subsets and their interaction, and thereby allow refined patient stratifications.

A notable strength of the study is the use of a well-annotated randomized RT cohort with long follow-up. Scoring was done by two independent raters and could rely on analyses of whole tissue sections rather than tissue microarray cores. Survival analyses are presented following a nonoptimized median-based dichotomization of cases into PDGFRb "low/high" groups. Interaction tests were also performed with continuous PDGFRb score as recommended (40). Future studies using optimized cutoff strategies would however be highly warranted. Such studies should be performed in populations where RT and control groups are well balanced regarding clinicopathologic characteristics to allow stringent separation between prognostic and predictive associations. Furthermore, validation cohorts should be selected to provide sufficient power for invasive IBE given their higher clinical relevance as compared with *in situ* recurrences. One notable limitation of the presented study remains the semiquantitative scoring procedure. A relevant task for future studies is the development of a standardized, automated scoring approach, ideally reporting staining intensity and positive stroma fraction separately. Stromal PDGFRb status has recently been determined using digital image analyses in tissue microarrays (23, 46, 47). Importantly, future studies should also explore if the response-predictive capacity of stromal PDGFRb act as an independent predictive factor as compared with the recently described DCISionRT test or the Oncotype DCIS score (17, 18, 48).

In summary, this study identifies low stromal PDGFRb as a candidate marker for RT benefit in DCIS. Validation studies, ideally analyzing *in situ* and invasive IBE separately, as well as elucidation of underlying biological mechanisms are prioritized topics for continued studies. These have the potential to advance the presented findings toward development of a novel biomarker of clinical utility and novel, more efficient combination treatments.

Authors' Disclosures

C. Strell reports other from PreludeDx outside the submitted work; in addition, C. Strell has a patent for biomarker pending. E. Holmberg reports other from PFS Genomics and other from Prelude Dx outside the submitted work; in addition, E. Holmberg has a patent for biomarker pending. P. Karlsson reports other from PFS Genomics and Prelude Dx outside the submitted work; in addition, P. Karlsson has a patent for biomarker pending. J. Bergh reports grants to Karolinska Institutet and/or University Hospital from Amgen, AstraZeneca, Bayer, Merck, Pfizer, Roche, and Sanofi-Aventis outside the submitted work, as well as payments from UpToDate to Asklepios Medicine HB for a chapter on prognostic and predictive factors in early, nonmetastatic breast cancer. T. Bremer reports personal fees and nonfinancial support from PreludeDx outside the submitted work; T. Bremer is CSO and founder of PreludeDx, a company developing radio-genomic tests to improve early-stage breast cancer treatment management. L.A. Akslen reports a patent for PDGFRb predictive for RT response in invasive breast cancer pending. A. Östman reports grants and personal fees from Eli Lilly and grants from IPSEN during the conduct of the study; in addition, A. Östman has a patent for biomarker pending. No disclosures were reported by the other authors.

Authors' Contributions

C. Strell: Conceptualization, data curation, formal analysis, supervision, methodology, writing–original draft, project administration, writing–review and editing. **D. Folkvaljon:** Data curation and methodology. **E. Holmberg:** Formal analysis. **A. Schiza:** Data curation. **V. Thurfjell:** Data curation. **P. Karlsson:** Resources, data curation, writing–review and editing. **J. Bergh:** Conceptualization, writing–review and editing. **T. Bremer:** Data curation, formal analysis, writing–review and editing. **L.A. Akslen:** Formal analysis and methodology. **F. Wärnberg:** Conceptualization, resources, writing–original draft, writing–review and editing. **A. Östman:** Conceptualization, data curation, supervision, funding acquisition, writing–original draft, writing–review and editing.

Role of the funders

This manuscript presents the own views of the authors, and is the product of professional research. The expressed views are not meant to represent the opinion of any entity the authors have been, are, or will be affiliated with or supported by.

Data Availability

The clinical database of the SweDCIS study including PDGFRb scores is available from the corresponding author on reasonable request.

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