

High expression of stromal PDGFR β is associated with reduced benefit of tamoxifen in breast cancer

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Abstract

Cancer-associated fibroblasts (CAFs) regulate tumour growth, metastasis and response to treatment. Recent studies indicate the existence of functionally distinct CAF subsets. Suggested mechanisms whereby CAFs can impact on treatment response include paracrine signalling affecting cancer cell drug sensitivity and effects on tumour drug uptake. PDGFR β is an important regulator of fibroblasts. Experimental studies have linked PDGFR β -positive fibroblasts to metastasis and also to reduced tumour drug uptake. This study has investigated the potential role of PDGFR β -positive fibroblasts in response to adjuvant tamoxifen treatment of breast cancer. Analyses of two breast cancer collections from randomised studies analysing adjuvant tamoxifen treatment in early breast cancer demonstrated significant benefit of tamoxifen in the group with low stromal PDGFR β , which was not observed in the group with high stromal PDGFR β . In general terms these findings provide novel evidence, derived from analyses of randomised clinical studies, of response-predictive capacity of a marker-defined subset of CAFs and, more specifically, identify stromal PDGFR β as a marker related to tamoxifen benefit in early breast cancer.

Keywords: breast cancer; tamoxifen; tumour stroma; PDGFR β

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Introduction

Cancer growth, metastasis and response to treatment are influenced by cells of the tumour microenvironment, including cancer-associated fibroblasts (CAFs) [1]. CAFs can modulate drug response by different mechanisms including effects on tumour physiology which regulate tumour drug uptake or paracrine signalling altering cancer cell drug sensitivity [2–4]. CAF-derived markers, such as caveolin, stromal phospho-Erk (pErk), and stroma-derived gene signatures have been linked to sensitivity to chemotherapy and endocrine treatment [5–7].

The PDGF family of growth factors, acting through PDGFR α and PDGFR β tyrosine kinase receptors, act as important regulators of CAFs [8,9]. Previous studies have demonstrated that high stromal PDGFR β is linked to shorter survival in population-based breast and prostate tumour collections [10,11]. Potential impact of PDGFR β -positive fibroblasts on drug sensitivity is suggested by mechanistic studies, which have demonstrated that PDGFR-signalling in fibroblasts can regulate treatment efficacy by controlling tumour drug uptake in a manner involving regulation of tumour interstitial fluid pressure [12,13].

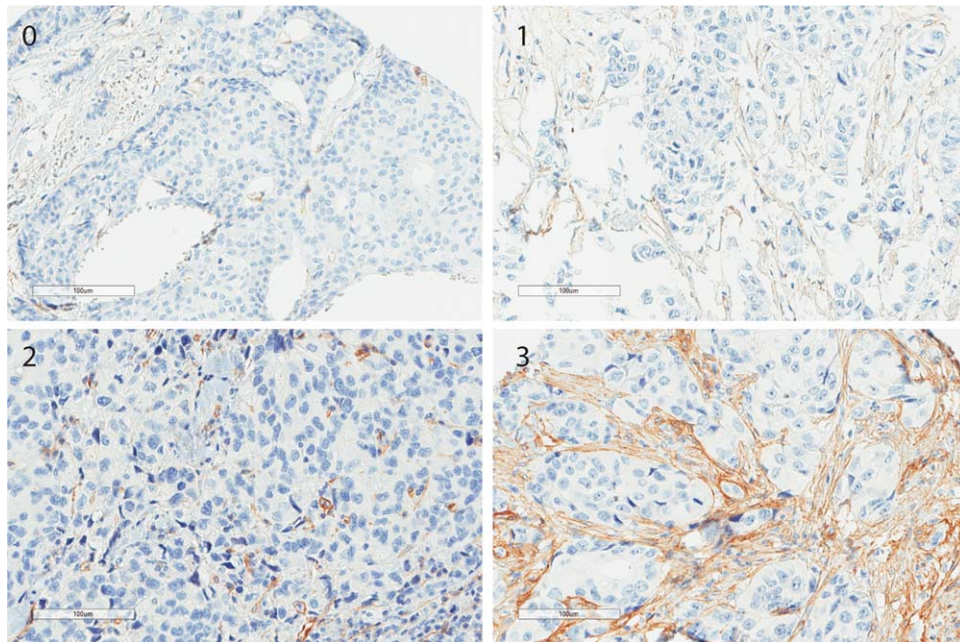


Figure 1. Tumour stromal PDGFR β immunohistochemical staining in the post-menopausal cohort. Upper left: Score 0. Upper right: Score 1. Lower left: Score 2. Lower right: Score 3. Scale bar 100 μ m.

Tamoxifen treatment represents a major component of clinical management of early breast cancer. Improved methods for identification of responsive patients remain a critical issue. Experimental and correlative studies have suggested a role for CAF-derived markers as biomarkers for tamoxifen benefit [14–16].

This study extends these earlier findings by analyses of the potential of stromal PDGFR β as a tamoxifen-sensitivity biomarker through analyses of two randomised study-derived breast cancer collections.

Material and methods

Cohort 1

Premenopausal patients with stage II (pT1pN1, pT2pN0, pT2pN1) primary breast cancer ($n = 564$) were randomised to 2 years of tamoxifen or no adjuvant treatment, in the SBII:2 multicentre trial [17]. Radiotherapy was delivered after breast conserving therapy and in patients with axillary lymph node metastases; chemotherapy and ovarian suppression was administered to <2% (nine patients). Median follow-up time was 13.6 years for patients without any event. Formalin fixed paraffin embedded blocks were retrieved from 500/564 patients and a tissue micro array (TMA) with two individual cores was constructed [17]. Assessment of ER, PR and HER2 were performed according to clinical protocols [18].

For ER- and PR-status the clinically used cut-point of more than 10% was used.

Cohort 2

The Stockholm tamoxifen trial included a cohort of 1780 postmenopausal breast cancer patients with node negative disease and a tumour size not exceeding 30 mm, randomised to 2 years of tamoxifen or no adjuvant treatment, irrespective of hormone receptor status. Radiotherapy was administered to patients receiving breast-conserving therapy. No adjuvant chemotherapy was given in this group of patients. The trial has previously been described in more detail [19]. TMAs with three individual cores were constructed from formalin fixed paraffin embedded tumours from 912 patients. The assessments of ER, PR and HER2 with immunohistochemistry have been previously described [20].

Immunohistochemistry

PDGFR β IHC for the pre-menopausal TMA series was performed as described earlier [21]. The post-menopausal TMA series was immunohistochemically stained for PDGFR β using the anti-PDGFR β antibody (#3169, 1:100 dilution, Cell Signaling Technology, USA) diluted in antibody diluent (Roche) in the Ventana system (Roche) with the Omnimap kit (5266548001, Roche). The secondary anti-rabbit antibody was used according to manufacturer's instructions (5269679001, Roche). For antigen retrieval high pH buffer was used (T6455, Sigma Aldrich). After

Table 1. Clinico-pathological characteristics and PDGFR β status in the pre- and post-menopausal cohorts

	Pre-menopausal patients stage II				Post-menopausal patients			
	<i>n</i>	PDGFR β <i>n</i> (%)		Significance	<i>n</i>	PDGFR β <i>n</i> (%)		Significance
		<3+	3+			<3+	3+	
All	360	127 (35)	233 (65)		All	528	306 (58)	222 (42)
Node status					Node status			
NO	108	34 (27)	74 (32)	<i>p</i> = 0.46	NO	528		
N+	251	93 (73)	158 (68)		N+	0		
Tumour size					Tumour size			
\leq 20 mm	128	44 (35)	84 (36)	<i>p</i> = 0.77	\leq 20 mm	385	213 (71)	172 (80)
>20 mm	231	83 (65)	148 (64)		>20 mm	132	88 (29)	44 (20)
ER status					ER status			
ER+	204	72 (65)	132 (70)	<i>p</i> = 0.41	ER+	393	221 (74)	172 (78)
ER-	97	39 (35)	58 (30)		ER-	127	78 (26)	49 (22)
PgR status					PgR status			
PgR+	203	67 (65)	136 (71)	<i>p</i> = 0.49	PgR+	236	136 (49)	100 (50)
PgR-	241	36 (35)	56 (29)		PgR-	241	140 (51)	101 (50)
HER2 status					HER2 status			
HER2-	226	77 (82)	149 (85)	<i>p</i> = 0.49	HER2-	420	245 (87)	175 (85)
HER2+	43	17 (18)	26 (15)		HER2+	69	38 (13)	31 (15)

staining in the Ventana autostainer samples were dehydrated in ethanol (70, 95, 99%) and xylene and mounted using PERTEX (00871, Histolab). TMA's were then scanned at the tissue profiling facility at SciLifeLab, Uppsala University and pictures taken with the Aperio ImageScope software (v.11.2.0.780, Leica Biosystems). Final scores (0–3) were derived from two independent readings from JP and CS blinded to outcome data. In cases of discordance between readings (around 10%) slides were re-visited for new consensus-scoring. Cohort 1 was made with two cores/tumour, whereas cohort 2 was made up of three cores/tumour. Mean-values for individual cores of each tumour were used for subsequent correlation and survival-analyses.

Statistical analyses

The association of PDGFR β with other clinicopathological factors was evaluated using the χ^2 -test. Time for follow-up was defined as the time from randomisation until the first event, loco-regional recurrence, distant recurrence, or death due to breast cancer. Survival curves and probabilities of recurrence-free survival (RFS) were estimated using the Kaplan-Meier method. Hazard ratios (HR) were calculated using Cox hazard regression analysis.

Results

Associations between stromal PDGFR β expression and clinico-pathological characteristics of early breast cancer

TMA's from tumours of two different randomised studies on tamoxifen benefit in pre- and post-

menopausal women [18,19], was subjected to PDGFR β IHC analyses and scored as previously described (Figure 1) [10,11].

High stromal PDGFR β expression was more common in the pre-menopausal group. In this group, 65% of cases displayed high stromal PDGFR β expression, whereas 42% of the post-menopausal cases displayed high levels of stromal PDGFR β expression (Table 1).

In the post-menopausal cohort a significant association (*p* = 0.023) was detected between high PDGFR β expression and small tumour size (Table 1). No significant association between stromal PDGFR β expression and clinico-pathological features were detected in the pre-menopausal group.

Impact of stromal PDGFR β expression on RFS in tamoxifen-treated ER+ breast cancer

A set of analyses, restricted to ER+ cases, were performed which compared treatment effects in pre- and post-menopausal subsets defined by stromal PDGFR β status.

As shown in Figure 2A, a significant benefit of tamoxifen treatment (*p* = 0.026), measured by Kaplan-Meier analyses of RFS, was detected in the low/moderate PDGFR β -expressing pre-menopausal group. Strikingly, this significant treatment benefit was not seen in the high PDGFR β expressing group. This differential effect of tamoxifen in the two marker-defined patient sub-groups was also seen in Cox regression analyses where treatment was associated with a significant HR in the low/moderate PDGFR β -expressing pre-menopausal group (HR = 0.40 (95% CI 0.18–0.90)), but not in the high PDGFR β expressing group (HR = 0.84 (95% CI 0.49–1.42)).

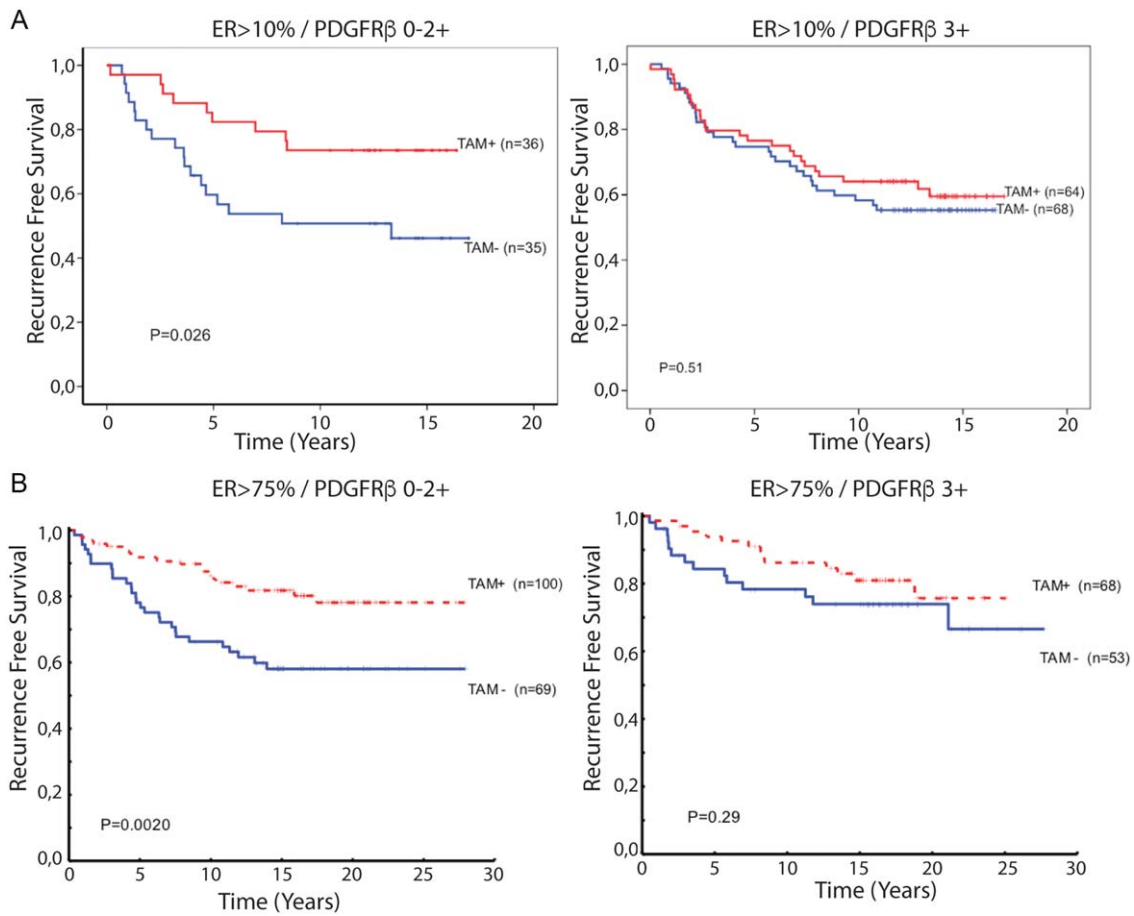


Figure 2. (A) Kaplan-Meier graphs showing recurrence free survival in the stromal PDGFRβ low/moderate (0–2+, left panel, RFS: HR = 0.40 (95% CI 0.18–0.90)) and high (3+, right panel, RFS: HR = 0.84 (95% CI 0.49–1.42)) groups treated or not with tamoxifen restricted to cases with more than 10% expression of ER in the pre-menopausal cohort. (B) Kaplan-Meier graphs showing recurrence free survival in the stromal PDGFRβ low/moderate (0–2+, left panel, RFS: HR = 0.41 (0.23–0.73)) and high (3+, right panel, RFS: HR = 0.67 (0.31–1.42)) groups treated or not with tamoxifen restricted to cases with >75% expression of ER in the post-menopausal cohort.

Initial analyses of the complete post-menopausal cohort yielded results with a trend of reduced tamoxifen benefit in the subset with high stromal PDGFRβ expression (data not shown). Based on findings from earlier meta-analyses that tamoxifen benefit is most prominent in cases with high ER expression novel analyses were performed on the subset of the post-menopausal cohort with more than 75% ER-positive cells (290 cases out of 393). Interestingly, analyses of this sub-group yielded results similar to those seen in the pre-menopausal cohort with significant tamoxifen-benefit, determined both by Kaplan-Meier analyses and Cox hazard regression analyses, detected in the PDGFRβ low/moderate group (HR = 0.41 (95% CI 0.23–0.73)), but not in the PDGFRβ high group (HR = 0.67 (95% CI 0.31–1.42)) (Figure 2B).

Together these analyses thus indicate that high stromal PDGFRβ is a marker for reduced benefit of tamoxifen.

Discussion

In contrast to the majority of studies analysing factors associated with benefit of tamoxifen this study describes previously un-recognised associations between a tumour stroma marker and tamoxifen benefit.

Support for the notion that stromal fibroblasts can impact on efficacy of drugs targeting malignant cells, have been presented from analyses of series of cases not derived from randomised studies [6,7]. The earlier analyses of the pre-menopausal cohort of the present study which identified pERK as a marker associated with tamoxifen efficacy, is to our knowledge the only other study which have demonstrated associations between a fibroblast-marker and treatment efficacy based on analyses of randomised studies [5]. The present findings thus represent a

significant addition in the efforts to translate and consolidate pre-clinical findings by analyses of well-annotated clinical samples.

The present study identifies associations between stromal PDGFR β and tamoxifen benefit. Earlier studies have shown that stromal PDGFR β status is largely independent from stroma abundance in general or stromal α -smooth muscle actin-positivity [10,22]. These findings therefore suggest that the detected association is not related to stroma abundance but rather reflects more specific biology of PDGFR β -positive stromal cells.

This study does not address if the detected association between stromal PDGFR β and tamoxifen benefit reflects a direct involvement of PDGFR β signalling in tamoxifen effects, or rather is related to other signalling effects of PDGFR β -positive stromal cells. Concerning the former, findings from model studies have demonstrated effects of stromal PDGFR β on tumour drug uptake [13,23,24]. Paracrine signalling from fibroblasts have also been shown to directly affect drug efficacy [2,25,26]. Previous experiments have indeed demonstrated tamoxifen-protective effects by co-cultured fibroblasts in tissue culture models [14–16]. According to preliminary studies this effect is not related to PDGFR β status of fibroblasts, since also fibroblast with down-regulation of PDGFR β displayed a protective effect (data not shown). The clinical associations therefore appear more likely to be related to PDGFR β -controlled drug exposure. Future studies could explore this possibility by measuring tamoxifen uptake, or ER activity, in tumour samples with known stromal PDGFR β status from tamoxifen treated cases.

Both cohorts represent randomised clinical trials with long time of follow-up, of importance as patients with ER-positive breast cancer frequently experience late relapses. With few exceptions the patients received no other systemic treatment than tamoxifen. A limitation is that the study is retrospective and at the time when the trials were implemented less women than today had breast conserving surgery. Type of surgery had however no influence on the results (data not shown).

Based on the results from the present study it seems highly appropriate to integrate fibroblast-related markers, in general, and PDGFR β , specifically, in future prospective efforts to identify tamoxifen-benefit biomarkers.

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Author contributions

All authors provided substantial contributions, were involved in preparation of the manuscript and approved the final version

Janna Paulsson: data collection, data analyses, manuscript writing; *Lisa Rydén*: conception of study, data analyses, manuscript writing; *Carina Strell*: data collection, data analyses; *Oliver Frings*: data analyses; *Nicholas P. Tobin*: data analyses; *Tommy Fornander*: conception of study; *Jonas Bergh*: manuscript writing; *Göran Landberg*: conception of study, manuscript writing; *Olle Stål*: conception of study, data analyses, manuscript writing; *Arne Östman*: conception of study, data analyses, manuscript writing.

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