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The prognostic significance of PELP1 expression in invasive breast cancer with emphasis on the ER-positive luminal-like subtype

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Abstract The transcription functions of oestrogen receptors (ER) are influenced by several coregulators such as PELP1 (proline, glutamate and leucine rich protein 1). The aim of the present study, which uses tissue microarrays and immunohistochemistry, is to explore the clinical and biological relevance of PELP1 protein expression in a large series of consecutive patients (1,162 patients) with invasive breast cancers with particular emphasis on its role in the ER-positive/luminal-like class of tumours. Our results showed that increased PELP1 expression is associated with tumours of larger size, higher histological grade, higher mitotic count, and with positive expression of basal cytokeratins (CK) (CK14; $P = 0.018$ and CK5/6; $P = 0.029$), P -cadherin ($P = 0.002$), p53 and MIB1 ($P = 0.018$). There was an inverse association between PELP1 expression and ER ($P = 0.002$), progesterone (PgR) ($P = 0.004$), androgen (AR) receptor ($P < 0.001$), and luminal CK (CK18; $P = 0.027$) expression. A significant association between

PELP1 expression and shorter breast cancer specific survival (BCSS) ($P = 0.002$) and disease-free survival (DFI) ($P = 0.006$) was found. Multivariate Cox hazard analysis showed that PELP1 expression was an independent predictor of shorter BCSS (Hazard ratio (HR) = 1.349, $P = 0.006$) and shorter DFI (HR = 1.255, $P = 0.011$). In the ER-positive/luminal-like group ($n = 768$), PELP1 expression showed similar association with other clinicopathological variables and was an independent predictor of shorter DFI (HR = 1.256, $P = 0.036$). In conclusion, PELP1 protein expression is an independent prognostic predictor of shorter BCSS and DFI in breast cancer and its elevated expression is positively associated with markers of poor outcome. PELP1 appears to have a potential application in assessing the clinical outcome of patients with ER-positive breast cancer.

Keywords Breast carcinoma · PELP1 ·
Oestrogen receptor · Prognosis · Immunohistochemistry ·
Tissue microarray

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Introduction

Oestrogen receptor (ER) plays an important role in breast cancer development, progression and response to therapy. The genomic and non-genomic functions of ER have highlighted the role of various ER co-regulators in the ER pathway. Subsequently, it is important to examine the status of the steroid receptor co-regulators to better understand the mechanisms of ER signalling and to identify their biological and clinical significance in breast cancer development.

PELP1 (proline, glutamate and leucine rich protein 1) is located on chromosome 17 [1]. It improves 17β -estradiol (E2) dependent transcriptional activation from the oestrogen

response element in a dose-dependent fashion and shows high expression in various tissues especially in the breast and brain. Importantly, PELP1 may add to the oncogenic properties of cancer cells by acting as a scaffolding protein that relates many signalling processes with ER through its interaction with other oncogenes including SRC, PI3K, STAT3, and EGFR [1].

Previous gene-knock-down studies of PELP1 have shown reduced E2 activation of AKT signalling pathway significantly and inhibited E2 genomic transcriptional effects on gene expression in breast cancer cells [2]. Regulation of aromatase by PELP1 represents a novel mechanism for autocrine oestrogen synthesis, which may lead to tumour proliferation [3]. These findings suggest an important tumourigenic role of PELP1 and may open a new targeted therapeutic approach by its inhibition [4].

Other studies suggest a different mechanism for the oncogenic properties of PELP1 through its involvement in histone remodelling. PELP1 maintains the balanced hypoacetylated state of histones, while ER binding reverses its role through hyperacetylation of histones through an unknown mechanism [5]. In addition, it has been suggested that PELP1 contributes to chromatin remodelling by affecting certain types of histone in cancer cells [6]. In a previous, breast cancer study, PELP1 expression was reported to be up-regulated in higher grade lymph node-positive invasive tumours [7], but the study did not specifically focus on PELP1 expression in ER+/luminal cancers. PELP1 protein expression was associated with tumour progression in other organs [8].

The value of PELP1 as a prognostic biomarker in defining breast cancer phenotypes remains undetermined. Therefore, the aim of the present study is to investigate the clinical relevance and biological relations of PELP1 protein expression in a large series of consecutive patients with invasive breast cancers, using high-throughput tissue microarrays (TMAs) and immunohistochemistry, and to test its association with other clinically and biologically relevant biomarkers. In addition we explored the PELP1 protein expression in the ER-positive patients' cohort.

Materials and methods

Patient selection and tissue microarray construction

Tissue microarrays were prepared from a series of primary operable breast carcinoma cases from consecutive patients aged 70 years or less presented to the Nottingham Breast Unit between 1988 and 1998 with tumours of less than 5 cm diameter on pre-operative measurement as previously reported [9]. This series is well characterised and contains patients' clinical and pathological data, including patients'

age, histologic tumour type, primary tumour size, lymph node status, mitotic count and histologic grade [10], Nottingham prognostic index (NPI) [11] and vascular invasion (VI). Survival data including development of recurrence, distant metastases (DM), survival time and disease-free interval (DFI) were maintained on a prospective basis. Breast cancer-specific survival (BCSS) was defined as the time in months from the date of the primary surgical treatment to the time of death from breast cancer. DFI was defined as the number of months from the date of the primary surgical treatment to the first loco regional relapse or distant metastasis. Mean follow-up time of this study was 124 months. Patient management was based on NPI and ER status as previously described [12].

Data on other biomarkers with strong relevance to breast cancer including oestrogen receptor (ER α), progesterone receptor (PgR), androgen receptor (AR), BRCA1, p53, FHIT, EGFR, HER2, HER3, HER4, E-cadherin, P-cadherin, basal and luminal cytokeratins (CKs) (CK5/6, CK14, CK18, CK19), neuroendocrine markers (Synaptophysin and Chromogranin A), cell cycle inhibitors (p21 and p27), p63, smooth muscle actin (SMA), MIB1, BCL2, FOXA1, Transferrin receptor (CD71), Thymidine kinase (TK1), and CARM1 protein expression were available [9, 12, 13].

Immunohistochemistry

Rabbit polyclonal antibody to PELP1 (NB100-1749; Novus Biologicals Inc., Littleton, CO, USA) was optimised at a working dilution of 1:100 using full-face sections of breast cancer excision tissue to assess the staining distribution. Immunohistochemical staining of PELP1 was performed on a set of full-face sections and the TMAs using a DakoCytomation Techmate 500 plus (DakoCytomation, Cambridge, UK) automatic immuno stainer with a labelled streptavidin biotin technique (LSAB) in accordance with the manufacturer's instructions and counter stained in haematoxylin as previously described [9]. Negative controls were performed by omitting the primary antibody and substitution with a diluent. Peptide blocking with PELP1 antigen (Novus Biologicals, NB100-1749PEP) was performed to verify the antibody specificity.

The H-score (histochemical score) was used to assess the intensity of staining and the percentage of stained cells [14]. Staining intensity was scored from 0, 1, 2, and 3, and the percentage of positive cells was determined for each score to produce a final score in the range 0–300. The cases were scored without the knowledge of the patient outcome.

The X-tile [15] programme was used to define optimal cut off points of PELP1 H-score values (<5, negative/low; ≥ 5 and <170, moderate; and ≥ 170 , strong expression). This programme randomly divides the total patient cohort into

two separate training and validation sets ranked by patient follow-up time. Statistical significance was tested by validating the obtained cut points to the validation set. The same programme was used to define optimal cut-off points for CARM1 expression (<30, negative/low; ≥ 30 and <150, moderate; and ≥ 150 , strong expression). For TK1, we used the median of the percentage of positive cells (8%) as a cut-off point.

HER2 scoring was performed using the Hercept test guidelines (DakoCytomation, Cambridge, UK).

Statistical analysis

Statistical analysis was performed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Association between PELP1 immunoreactivity and different clinicopathological parameters was studied using chi-square test. Standard cut-off values for the different biomarkers, needed to determine categorical scores before statistical analysis, were the same as those published in previous studies [9, 12, 16]. Survival curves were estimated by the Kaplan–Meier method with a log rank test to assess their significance. Patients who died from reasons other than breast cancer were censored during survival analysis. Multivariate Cox proportional hazard regression models were used to evaluate any independent prognostic effect of the variables with 95% confidence interval. A *P*-value of <0.05 was considered significant.

This study was approved by the Nottingham Research Ethics Committee 2 under the title “Development of a molecular genetics classification of breast cancer”.

Results

After excluding the uninformative TMA cores from the study, 1,162 tumours were available for assessment. The median age of the patients was 55 years (range 27–70). Sixty-eight percent of patients had tumours greater than or equal to 1.5 cm in size. Fifty-nine percent of the tumours were ductal of no special type, 17% of the tumours were grade 1, and 27.8% showed good NPI. Thirty percent of the patients developed metastatic disease during follow-up, and 41.7% developed tumour recurrence. Patients' characteristics are summarised in Table 1.

PELP1 staining was detected in the nuclei of the malignant cells as well as in some luminal ductal epithelial cells of associated normal tissues in the cores. Applying the peptide blocking successfully abrogated staining (Fig. 1a, b). In the whole series, 17.2% of the tumours showed negative or low expression, 69.3% showed moderate expression (Fig. 1c, d), and 13.5% showed strong expression (Fig. 1e, f). No cytoplasmic staining was observed.

Table 1 Patients' characteristics

Variable	Number (%)
Patients' age	
<40	87 (7.5)
40–50	331 (28.5)
51–60	394 (33.9)
>60	350 (30.1)
Tumour size (cm)	
≤ 1.5	371 (31.9)
>1.5	791 (68.1)
Lymph node stage	
1	701 (60.5)
2	353 (30.5)
3	104 (9)
Tumour grade	
1	198 (17.1)
2	366 (31.6)
3	596 (51.3)
Nottingham prognostic index (NPI)	
Poor	202 (27.8)
Moderate	637 (54.8)
Good	323 (17.4)
Distant metastasis (DM)	
No	796 (69.3)
Positive	353 (30.7)
Recurrence	
No	662 (58.3)
Positive	474 (41.7)
Vascular invasion (VI)	
No	644 (55.4)
Probable	125 (10.8)
Definite	390 (33.8)
Histologic tumour type	
Ductal/NST	688 (59.2)
Lobular	135 (11.7)
Tubular and Tubular mixed	230 (19.8)
Medullary	30 (2.6)
Other special types ^a	18 (1.5)
Mixed ^b	61 (5.2)
Menopausal status	
Premenopausal	435 (37.4)
Postmenopausal	727 (62.6)

^a Includes mucoid, invasive cribriform and invasive papillary carcinoma

^b Includes ductal/NST mixed with lobular or special types

Correlation of PELP1 protein expression with other histopathological variables

In the whole series of unselected breast cancer patients, increased PELP1 expression was associated with markers

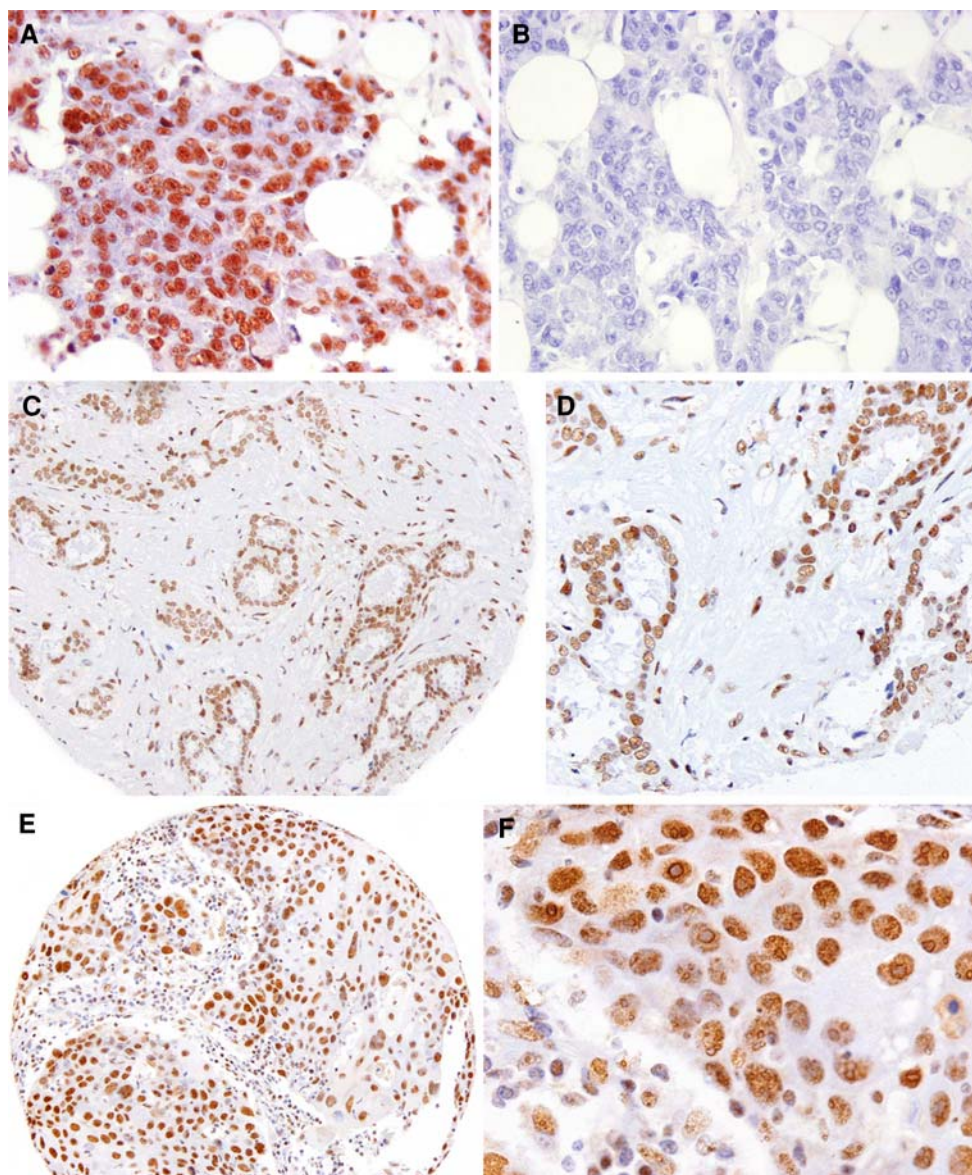


Fig. 1 Grade III infiltrating ductal carcinoma NST showing strong PELP1 nuclear expression. PELP1 nuclear staining was lost with application of the peptide blocking, used as a negative control (**a**, **b** $\times 400$). TMA core of a low grade ductal carcinoma showing moderate

positive PELP1 nuclear expression (**c** $\times 100$, **d** $\times 200$). TMA core of a high grade ductal carcinoma showing strong positive PELP1 nuclear expression (**e** $\times 100$, **f** $\times 400$)

of poor prognosis such as larger primary tumour size, higher grade tumours with raised mitotic count ($P = 0.004$) and with the poor NPI group. It also showed an association with histologic tumour type with frequent expression in the poor prognostic group [ductal/NST ($P = 0.029$)] (Table 2). No association was found between PELP1 and patients' age, lymph node stage, vascular invasion and menopausal status. When the analysis was repeated on ER-positive/luminal-like group of tumours ($n = 768$), PELP1 expression showed significant positive association with larger tumour size and development of tumour recurrence ($P = 0.027$) (Table 3).

Correlation of PELP1 protein expression with other biomarkers

In the whole series, we found a positive association between PELP1 expression and biomarkers of poor prognosis, including basal CKs (CK14, $P = 0.018$; and CK5/6; $P = 0.029$), *P*-cadherin, P53, MIB1 ($P = 0.018$), TK1 ($P = 0.002$) and CARM1 ($P < 0.001$) expression. An inverse association was found between PELP1 expression and ER α , PgR, AR, and luminal CK18 expression. No association was found between PELP1 and other biomarkers included in the study (Table 4).

Table 2 Relation of PELP1 expression to other clinicopathological parameters in the whole series of breast cancer patients

Variable	PELP1 expression			χ^2	P- value
	Low	Moderate	Strong		
Patients' age				3.069	0.8
<40	14	61	12		
40–50	55	231	45		
51–60	78	264	52		
>60	53	249	48		
Tumour size (cm)				11.098	0.004
≤1.5	80	254	37		
>1.5	120	551	120		
Lymph node stage				0.930	0.920
1 (negative)	118	491	92		
2 (1–3 LN)	63	238	52		
3 (>3 LN)	18	73	13		
Tumour grade				10.045	0.040
1	44	128	26		
2	73	247	46		
3	83	428	85		
NPI				14.045	0.007
Poor	35	137	30		
Moderate	91	451	95		
Good	74	217	32		
DM				6.873	0.032
No	145	554	97		
Positive	49	244	60		
Recurrence				11.895	0.003
No	133	451	78		
Positive	62	336	76		
VI				0.593	0.964
No	111	450	83		
Probable	23	85	17		
Definite	66	268	56		
Histologic tumour type				19.987	0.029
Ductal/NST	109	482	97		
Lobular	33	89	13		
Tubular and tubular mixed	42	156	32		
Medullary	1	22	7		
Other special types ^a	7	10	1		
Mixed ^b	8	46	7		
Mitosis				15.465	0.004
1	88	257	48		
2	37	137	27		
3	68	388	79		
Menopausal status				1.598	0.450
Premenopausal	67	308	60		
Postmenopausal	133	497	97		

^a Includes mucoid, invasive cribriform and invasive papillary carcinoma^b Includes ductal/NST mixed with lobular or special types**Table 3** Relation of PELP1 expression to other clinicopathological parameters in the ER-positive/luminal-like cohort of breast cancer patients

Variable	PELP1 expression			χ^2	P- value
	Low	Moderate	Strong		
Patients' age				8.354	0.213
<40	5	31	3		
40–50	34	139	24		
51–60	63	175	27		
>60	45	184	38		
Tumour size (cm)				6.945	0.031
≤1.5	63	189	24		
>1.5	84	340	68		
Lymph node stage				1.109	0.893
1 (negative)	89	322	51		
2 (1–3 LN)	46	167	33		
3 (>3 LN)	11	38	8		
Tumour grade				4.362	0.359
1	37	111	22		
2	67	217	35		
3	43	200	35		
NPI				6.379	0.173
Poor	19	69	16		
Moderate	64	267	50		
Good	64	193	26		
DM				2.624	0.269
No	108	375	60		
Positive	36	151	32		
Recurrence				7.222	0.027
No	100	305	47		
Positive	45	216	42		
VI				4.109	0.392
No	82	298	43		
Probable	19	60	16		
Definite	46	169	33		
Histologic tumour type				10.585	0.391
Ductal/NST	69	269	49		
Lobular	29	82	11		
Tubular and tubular mixed	38	129	26		
Medullary	0	3	0		
Other special types ^a	5	8	0		
Mixed ^b	6	38	6		
Mitosis				8.124	0.087
1	80	229	38		
2	29	108	19		
3	33	175	32		
Menopausal status				3.488	0.175
Premenopausal	38	178	27		
Postmenopausal	109	351	65		

^a Includes mucoid, invasive cribriform and invasive papillary carcinoma^b Includes ductal/NST mixed with lobular or special types

Table 4 Relation of PELP1 expression to other biomarkers in the whole series of breast cancer patients

Variable	PELP1 expression			χ^2	P-value
	Low	Moderate	Strong		
CK 5/6				7.090	0.029
Negative	168	653	119		
Positive	23	128	35		
CK 14				8.090	0.018
Negative	174	669	121		
Positive	16	97	58		
CK18				6.262	0.044
Negative	15	109	25		
Positive	163	619	121		
CK19				2.393	0.302
Negative	16	76	20		
Positive	175	705	131		
ER				12.108	0.002
Negative	40	236	58		
Positive	147	529	92		
PgR				11.009	0.004
Negative	58	343	70		
Positive	123	420	79		
AR				16.078	<0.001
Negative	42	279	60		
Positive	136	449	81		
P53				9.372	0.009
Negative	150	545	106		
Positive	33	217	47		
FHIT				1.575	0.455
Negative	37	122	24		
Positive	133	567	113		
BRCA1				4.882	0.087
Negative	21	110	12		
Positive	142	552	114		
Bcl2				10.961	0.090
Negative	28	134	26		
Weak	18	113	20		
Moderate	53	162	20		
Strong	13	46	6		
MIB1				8.033	0.018
Low	53	163	20		
High	47	218	45		
P-Cad				12.588	0.002
Negative	96	295	53		
Positive	68	362	80		
E-Cad				1.240	0.538
Negative	69	312	57		
Positive	120	455	92		

Table 4 continued

Variable	PELP1 expression			χ^2	P-value
	Low	Moderate	Strong		
FOXA1				0.378	0.828
Negative	73	334	64		
Positive	72	298	55		
Chromogranin A				3.234	0.198
Negative	160	646	116		
Positive	11	55	16		
Synaptophysin				0.881	0.644
Negative	159	644	130		
Positive	13	50	7		
HER2				5.633	0.060
Negative	175	664	134		
Positive	15	111	19		
HER3				1.908	0.385
Negative	18	69	9		
Positive	152	583	125		
HER4				5.845	0.054
Negative	38	117	33		
Positive	121	548	100		
EGFR				1.813	0.404
Negative	134	558	103		
Positive	31	135	33		
p63				1.376	0.503
Negative	186	760	148		
Positive	3	15	5		
Smooth muscle actin				1.218	0.544
Negative	166	660	128		
Positive	23	108	25		
p21				0.492	0.782
Negative	73	370	70		
Positive	66	296	60		
p27				3.218	0.200
Negative	88	425	71		
Positive	29	161	38		
CARM1				60.987	<0.001
Low	75	200	21		
Moderate	49	366	70		
Strong	20	105	45		
CD71				4.310	0.116
Negative	75	306	49		
Positive	78	405	84		
Thymidine kinase 1 (TK1)				12.344	0.002
Low	79	261	31		
High	60	286	62		

In ER-positive/luminal-like group of tumours, PELP1 expression was associated with AR expression ($P = 0.021$), FHIT ($P = 0.028$), TK1 ($P = 0.011$) and CARM1 ($P < 0.001$) expression. However, when the ER-negative group was separately assessed, no association was found between PELP1 protein expression and any of the clinicopathological variables included in this study apart from its association with positive *P*-cadherin expression ($P < 0.001$).

Patients' outcome

Breast cancer specific survival

In the whole patient series, an association between PELP1 expression and shorter BCSS was found (log rank (LR) = 12.168, $P = 0.002$) (Fig. 2a). Multivariate Cox hazard analysis including tumour size, histologic grade, lymph node stage, vascular invasion, ER expression showed that PELP1 expression was an independent predictor of shorter

BCSS (Hazard ratio (HR) = 1.349, $P = 0.006$, 95%CI = 1.091–1.668).

In a univariate analysis of ER-positive cohort, PELP1 expression also showed an association with shorter BCSS (LR = 7.029, $P = 0.030$) (Fig. 2c). However, in multivariate Cox analysis of ER-positive cohort, PELP1 was not an independent predictor of BCSS (HR = 1.302, $P = 0.061$, 95% CI = 0.987–1.717) (Table 5).

Disease-free interval

In the whole patient series, an association between PELP1 expression and shorter DFI was found (LR = 10.336, $P = 0.006$) (Fig. 2b). Multivariate Cox hazard analysis showed that PELP1 expression was an independent predictor of shorter DFI (HR = 1.255, $P = 0.011$, 95% CI = 1.053–1.495).

In the ER-positive cohort, PELP1 expression showed an association with shorter DFI (LR = 6.805, $P = 0.033$) in

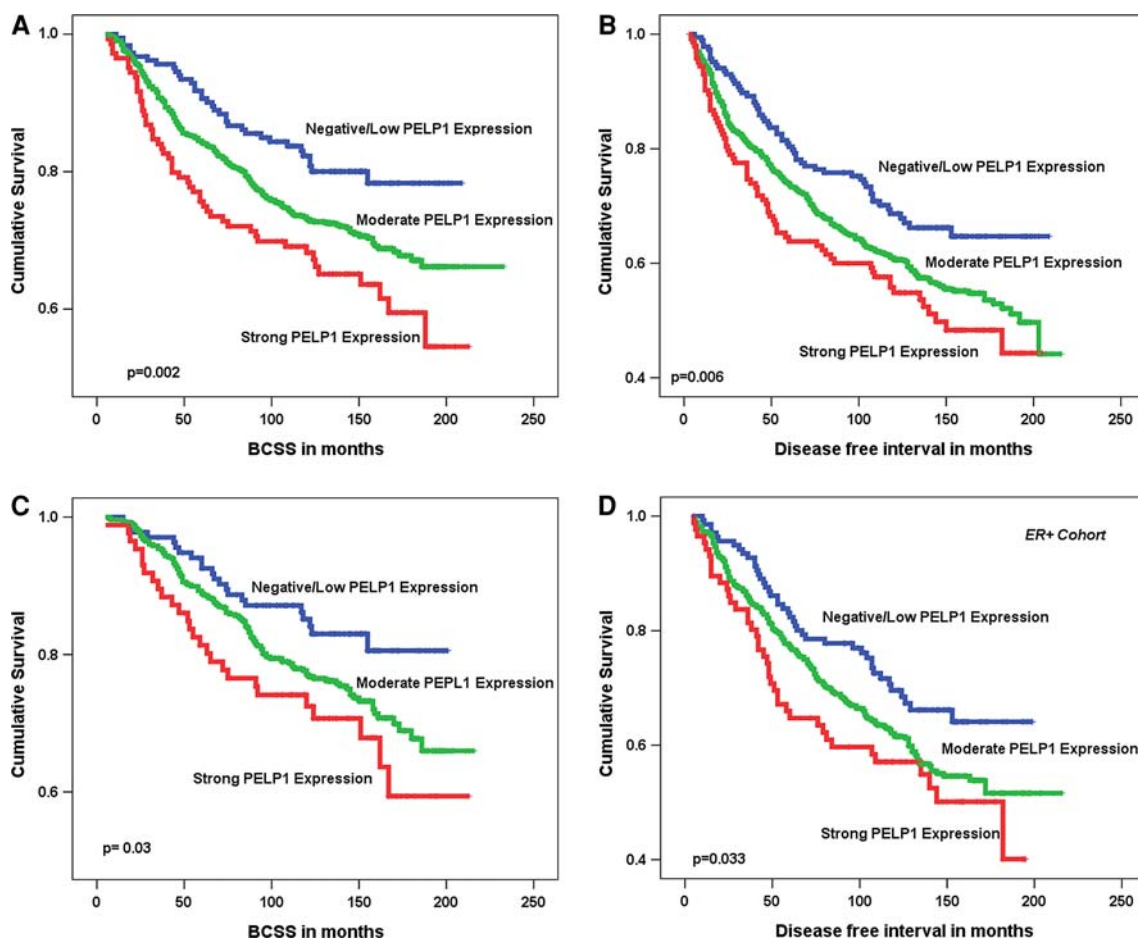


Fig. 2 a Kaplan–Meier plot of PELP1 expression in the whole series of unselected breast cancer patients with respect to BCSS. b Kaplan–Meier plot of PELP1 expression in the whole series of unselected breast cancer patients with respect to DFI. c Kaplan–Meier plot of

PELP1 expression in ER-positive cohort with respect to BCSS. d Kaplan–Meier plot of PELP1 expression in ER-positive cohort with respect to DFI

Table 5 Multivariate COX regression model for predictors of BCSS in (A) the whole patient cohort and (B) ER-positive patient cohort

Variable	P value	HR	95% CI	
			Lower	Upper
(A) Whole patient cohort				
PELP1 expression	0.006	1.349	1.091	1.668
ER expression	0.104	0.808	0.625	1.045
Tumour size	0.005	1.602	1.155	2.223
Tumour stage	<0.001	1.893	1.592	2.251
Tumour grade	<0.001	1.724	1.386	2.145
Vascular invasion	0.002	1.240	1.084	1.420
(B) ER-positive patient cohort				
PELP1 expression	0.061	1.302	0.987	1.717
Tumour size	0.009	1.718	1.144	2.581
Tumour stage	<0.001	1.775	1.413	2.229
Tumour grade	<0.001	1.885	1.474	2.412
Vascular invasion	0.001	1.339	1.125	1.594

Table 6 Multivariate COX regression model for predictors of DFI in (A) the whole patient cohort and (B) ER-positive patient cohort

Variable	P value	HR	95% CI	
			Lower	Upper
(A) The whole patient cohort				
PELP1 expression	0.011	1.255	1.053	1.495
ER expression	0.462	0.920	0.735	1.150
Tumour size	0.093	1.225	0.966	1.553
Tumour stage	<0.001	1.710	1.471	1.988
Tumour grade	0.002	1.279	1.092	1.498
Vascular invasion	0.002	1.192	1.067	1.331
(B) ER-positive patient cohort				
PELP1 expression	0.036	1.256	1.015	1.553
Tumour size	0.090	1.273	0.963	1.682
Tumour stage	<0.001	1.547	1.282	1.867
Tumour grade	<0.001	1.372	1.150	1.636
Vascular invasion	0.005	1.211	1.058	1.386

univariate analysis (Fig. 2d) as well as in multivariate analysis (HR = 1.256, $P = 0.036$, 95% CI = 1.015–1.553) (Table 6).

Discussion

Recently, gene expression profiling studies of breast cancer have identified specific molecular subtypes with clinical and biological implications [17–22]. Importantly, ER status has been found to be a defining marker of molecular assignment, supporting the fact that ER-positive and

ER-negative breast cancers are two different entities. It is recognised that ER-positive tumours, which comprise the majority of breast cancer cases, are a heterogeneous group of tumours with variable outcome and response to therapy [9, 19, 21, 23, 24]. These studies have classified ER-positive/luminal like tumours into different biological classes. The so-called Luminal A subtype has higher expression of ER-related genes and lower expression of proliferative genes than the luminal B subtype [20]. However, the number of subclasses within the luminal group and their precise definition remains uncertain and varies between published series. This has raised the need to discover candidate biomarkers to refine the subclassification of this important breast cancer subtype. To date, PELP1 has not been identified as a discriminating marker in the luminal subclasses of breast cancer. Our results, as discussed below, imply that PELP1 has the potential to stratify patients with ER-positive breast cancer into biological subclasses with differing prognoses.

In this study, the status of the steroid ER co-regulator PELP1 was investigated in a large cohort of patients with breast cancer to better understand its clinical and biological significance. We found a positive association between PELP1 and known features of poor prognosis and aggressive tumour behaviour including larger tumour size, higher histological grade, frequent development of distant metastasis, and tumour recurrence in the whole patient series as well as in the ER-positive cohort. These findings support the emerging data that PELP1 interacts with many proteins and activates several oncogenes that are related to the aggressive tumour characteristics and metastatic behaviour, including SRC, phosphatidylinositol 3 kinase (PI3 K), and signal transducers and activators of transcription 3 (STAT3) [25].

In this study, we found a significant positive correlation between PELP1 and CRAM1 which is necessary for the E2-induced proliferation of breast cancer cells via E2F1 and its target genes [26, 27]. This positive correlation at the protein level suggests a possible synergistic action between PELP1 and CARM1, being both ER coactivators, in E2-induced proliferation of ER-positive breast cancer cells.

The significance of genomic and non-genomic ER activity in mediating oestrogen signalling to promote cell proliferation and survival in breast cancer cells has been documented [28]. Many studies have highlighted the importance of PELP1 in tumour progression through increasing E2-mediated cell proliferation possibly through its requirement to ER alpha interaction with SRC which leads to the activation of MAPK pathway [29]. Our data implicate the involvement of PELP1 in tumour proliferation as we identified elevated expression in highly proliferative tumours, assessed by MIB1, TK1, mitotic count, and also notable elevation in high-grade tumours.

Supporting its poor prognostic role, we found a significant positive association between PELP1 and expression of basal CKs, *P*-Cadherin and p53, which are more frequently expressed in basal-like breast cancer and are associated with poor prognosis. As expected, we found an inverse relation between luminal CK and steroid receptor expression, which are markers of good prognosis in breast cancer.

A key aim of this study was to assess the prognostic ability of PELP1 in ER-positive/luminal-like breast cancer patients. In this important group of patients, we found that PELP1 expression is significantly associated with shorter BCSS and shorter DFI, which implies its role in subclassification of ER-positive groups into prognostic subgroups.

In conclusion, PELP1 expression is an independent prognostic factor of shorter survival in breast cancer, and its elevated expression is positively associated with markers of poor prognosis. The results of this study demonstrate the biological and prognostic role of PELP1 in breast cancer, which cannot be considered as a mere reflection of ER expression as evidenced by its role in the whole series of breast cancer as well as in the ER-positive/luminal-like subclass. This study suggests that PELP1 protein expression in breast cancer could have a role in clinical decision making and assessment of prognosis, particularly in the ER-positive luminal class. Furthermore, improved understanding of the functional role of PELP1 and its mechanism of action in breast may reveal a role as a therapeutic target.

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