Supplemental TABLE 1

Characteristics of our series of invasive breast tumors (n = 1432) assessed by immunohistochemistry for GRPR expression

Characteristic	Category	%	No. of Patients
Age	≤ 40 y	5.9	8
	40 - 50 y	20.1	287
	> 50 y	74.0	1060
Histology	IDC	85.3	1221
	ILC	11.2	161
	Other	3.5	50
Pathological size	<20mm	67.1	961
	≥20mm	32.9	471
SBR grade	I	25.0	358
	II	46.2	661
	III	27.7	397
	Missing information	1.1	16
Ki-67	<20%	77.9	1115
	≥20%	22.1	317
Lymph node status	N_0	59.2	848
	N^+	33.0	472
	N_x	7.8	112
Estrogen receptor	0%	10.5	150
	≥1%	89.5	1282
Progesterone receptor	0%	18.6	266
	≥1%	81.4	1166
Androgen receptor	<10%	12.8	184
	≥10%	87.2	1248
HER2 overexpression	No	89.8	1286
	Yes	10.2	146
Phenotype	Luminal A	67.2	963
	Luminal B HER2-	15.4	220
	Luminal B HER2+	6.9	99
	HER2-enriched	3.3	47
	Triple-negative	7.2	103

Abbreviations: GRPR: gastrin-releasing peptide receptor; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; SBR: Scarff-Bloom-Richardson; HER2: human epidermal growth factor receptor-2.

Supplemental TABLE 2

Primary antibodies and procedural specifications

Primary antibody	Type	Provider, code	Dilution	Pretreatment	Incubation time	Revelation
Anti-GRPR	Rabbit polyclonal	Acris Antibody, SP4337P	1/1400	Protease-1	52 min	Ultra View™ universal DAB detection kit
Anti-ER	Rabbit monoclonal	Roche Diagnostics, SP1	Prediluted	CC1 standard	32 min	Ultra View™ universal DAB detection kit
Anti-PR	Rabbit monoclonal	Roche Diagnostics, 1E2	Prediluted	CC1 court	12 min	Ultra View™ universal DAB detection kit
Anti-HER2	Rabbit monoclonal	Roche Diagnostics, 4B5	Prediluted	CC1 court	12 min	Ultra View™ universal DAB detection kit
Anti-AR	Rabbit monoclonal	Roche Diagnostics, SP107	Prediluted	CC1 standard	32 min	Ultra View™ universal DAB detection kit
Anti-Ki-67	Rabbit monoclonal	Roche Diagnostics, (30-9)	Prediluted	CC1 standard	32 min	Ultra View™ universal DAB detection kit

Abbreviations: GRPR: gastrin-releasing peptide receptor; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor-2; AR: androgen receptor. CC1: cell conditioning 1

Supplemental TABLE 3

Comparison of GRPR expression in human breast cancer cell lines with western blotting and immunochemistry

Cell lines	GRP-R expression	
	WB	ICC Holland-fixed
MCF-7	Strong	Strong/Moderate
MDA-453	Weak	Weak
MDA-468	Weak	Weak
SKBR3	Moderate	Moderate
T47-D	Weak	Weak
ZR75.1	Strong	Strong
Positive control (Langerhans islets)	Strong+++	Strong+++
Negative control (Human placenta)	Weak/Absent	Absent

Abbreviations: WB: western blot; ICC: immunocytochemistry.

Supplemental TABLE 4

Distribution of low and high GRPR expression within different categories of breast cancer patients with ER-negative tumors

Characteristic	Category	No. of Patients	GRPR expression Low %	GRPR expression High %	*P*-value
Age	≤ 40y	20	100.0	0.0	*P*= 0.13
	> 40y	130	86.2	13.8	
Histology*	IDC	141	88.7	11.3	*P*= 0.32ǂ
	ILC	3	66.7	33.3	
SBR grade	I + II	20	90.0	10.0	*P*=1ǂ
	III	130	87.7	12.3	
Ki-67	< 20%	43	94.7	5.3	*P*= 0.64
	≥ 20%	107	88.8	11.2	
Lymph node status*	N_0	86	88.4	11.6	*P*= 0.94
	N^+	58	87.9	12.1	
Androgen receptor	< 10%	93	92.5	7.5	*P*= 0.03
	≥ 10%	57	80.7	19.3	
Pathological size	< 20mm	78	88.5	11.5	*P*= 0.87
	≥ 20mm	72	87.5	12.5	
HER2 overexpression	Yes	47	78.7	21.3	*P*= 0.018
	No	103	92.2	7.8	

* Patients with other histology "miscellaneous" are excluded from statistical analysis. Number of patients with missing information regarding items: lymph node status (N_x), n=6.

Statistical analysis based on χ^2 or Fisher testǂ; $P < 0.05$ was considered significant.

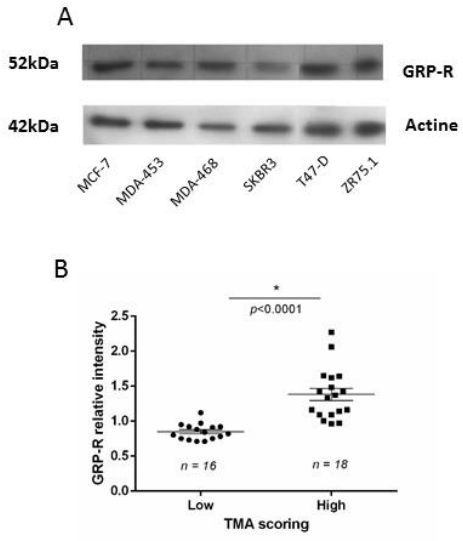
Abbreviations: ER: estrogen receptor; GRPR: gastrin-releasing peptide receptor; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; HER2: human epidermal growth factor receptor-2; SBR: Scarff-Bloom-Richardson

Supplemental TABLE 5

Univariate and multivariate analysis of prognostic factors for distant metastasis-free interval (DMFI) of patients with ER-positive tumors

					Univariate analysis		Multivariate analysis	
Characteristic	Category	No. of Patients	Events (n)	Events (%)	Hazard ratio [95%CI]	*P*	Hazard ratio [95%CI]	*P*
GRPR expression	Low	215	32	14.9	1	0.0369	Not retained	
	High	1067	97	9.1	0.6 [0.4-1.0]			
Histological subtype	IDC	1080	109	10.1	1	0.8831		
	ILC	158	17	10.8	1.1 [0.6-1.8]	0.8238		
	Others	38	3	7.9	1.3 [0.4-4.1]	0.6464		
Age	> 40y	1217	120	9.9	1	0.3344		
	≤ 40 y	65	9	13.8	1.4 [0.7-2.7]			
Pathological size	<20mm	883	56	6.3	1	<0.0001	1	<0.0001
	≥20mm	399	73	18.3	3.2 [2.3-4.6]		2.2 [1.5-3.2]	
SBR grade	I	356	11	3.1	1	<0.0001	1	0.0039
	II	643	72	11.2	3.9 [2.1-7.4]	<0.0001	3.0 [1.6-5.7]	0.0009
	III	267	45	16.9	5.9 [3.1-11.5]	<0.0001	2.6 [1.2-5.4]	0.0113
Lymph node status	N_0	762	49	6.4	1	<0.0001	1	0.0002
	N^+	414	74	17.9	3.1 [2.2-4.4]	<0.0001	2.2[1.5-3.2]	<0.0001
	Missing	106	6	5.7	1.0 [0.4-2.4]	0.9552	1.3[0.6-3.1]	0.5284
Progesterone receptor	0%	116	18	15.5			Not retained	
	≥1%	1166	111	9.5	0.6 [0.4-1.0]	0.0374		
Androgen receptor	<10%	91	15	16.5	1		Not retained	
	≥10%	1191	114	9.6	0.6 [0.3-0.9]	0.0298		
HER2 over-expression	No	1183	116	9.8			Not retained	
	Yes	99	13	13.1	1.3 [0.7-2.3]	0.3344		
Ki-67	< 20%	1072	86	8.0	1		1	0.0029
	≥ 20%	210	43	20.5	2.6 [1.8-3.8]	<0.0001	1.9 [1.3-3.0]	

Statistical analysis based on a Cox model; $P < 0.05$ was considered significant. Abbreviations: AR: androgen receptor; ER: estrogen receptor; GRPR: gastrin-releasing peptide receptor; IDC: invasive ductal carcinoma; ILC: invasive lobular carcinoma; HER2: human epidermal growth factor receptor-2; SBR: Scarff-Bloom-Richardson



Supplemental FIGURE 1

Validation steps for GRPR immunohistochemistry. (A) Western Blot of GRPR in human breast cancer cell lines: Analysis of western blots revealed that a single band is visible at the expected GRPR molecular weight. The study of various human breast cancer cell lines showed a strong expression in MCF-7 (ER^+, $PR^{\pm}$, $HER2^-$) and ZR75.1 (ER^+, $PR^{\pm}$, $HER2^{\pm}$) cell lines and a moderate expression in the SKBR3 (ER^-, PR^-, $HER2^+$) cell line. MDA-MB-453 (ER^-, PR^-, $HER2^+$, RA^+), MDA-MB-468 (ER^-, PR^-, $HER2^-$) and T47D (ER^+, $PR^{\pm}$, $HER2^-$) cell line express low level of GRP-R. (**B)** Correlation of GRPR signal recorded in immunohistochemistry (abscissa) and Western Blot (ordinate) of corresponding frozen tumors from 34 patients. All samples had greater than 50% of tumor cells. Immunohistochemistry scoring was compared with western blotting semi-quantification. Tumors with low GRPR expression in IHC showed the lowest GRPR expression in western blot. Again, tumors with high expression in IHC had the highest GRPR expression in western blot. The primary antibody anti-GRPR could statistically discriminate low GRPR-expressing tumors from high-GRPR expressing tumors ($P < 0.001$).