Journal of Oncological Science 2 (2016) 7–11

Contents lists available at ScienceDirect

Journal of Oncological Science

journal homepage: https://www.elsevier.com/locate/jons

Original article

Clinical significance of serum protease-activated receptor 1 (PAR1) level in patients with breast cancer

Rumeysa Ciftci [a, *], Faruk Tas [b, 1], Elif Bilgin [c, 2], Serkan Keskin [b, 3], Ibrahim Yildiz [b, 4], Derya Duranyildiz [c, 5], Pınar Saip [b, 6], Hasan Karanlik [d, 7], Sezai Vatansever [b, 8]

[a] Bakirkoy Dr Sadi Konuk Education and Research Hospital, Medical Oncology Department, Istanbul, Turkey
[b] Istanbul University, Institute of Oncology, Medical Oncology Department Capa, Istanbul, Turkey
[c] Istanbul University, Institute of Oncology, Basic Oncology Department Capa, Istanbul, Turkey
[d] Istanbul University, Institute of Oncology, Surgical Oncology Unit, Capa, Istanbul, Turkey

ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form
21 March 2016
Accepted 20 April 2016
Available online 2 July 2016

Keywords:
Breast cancer
Serum PAR1 level
Survival

ABSTRACT

Background: The protease-activated receptor 1 (PAR1) is associated with increased invasiveness of breast cancer (BC) cell lines and its overexpression is significantly correlated with advanced stage and poor prognosis. The aim of this study was to determine the clinical significance of the serum PAR1 levels in BC patients.
Methods: We enrolled 96 female patients with pathologically diagnosed BC who did not receive chemotherapy (CT) or radiotherapy. Serum PAR1 levels were measured by ELISA method and compared with 30 healthy controls.
Results: The mean serum PAR1 level of BC patients was significantly higher than controls (3.07 vs 2.82 ng/ml, $p = 0.011$). The levels of PAR1 tended to be higher among CT responders ($p = 0.052$) and grade I disease than others ($p = 0.055$). However, there was no significant difference in PAR1 levels according to other clinic-pathological or laboratory parameters. Serum PAR1 level did not have a significant impact on overall survival in both univariate ($p = 0.73$) and multivariate analysis ($p = 0.67$).
Conclusion: Serum PAR1 level is elevated in BC patients and may have predictive role for CT response. However, it has no prognostic role on survival.

1. Introduction

Despite all developments in screening and treatment interventions, breast cancer (BC) is the most common and the second mortal cancer type among women in worldwide.[1] In addition, BC is a very heterogeneous disease. Therefore, new markers which have diagnostic, predictive or prognostic value could provide a better classification of patients and guide the treatment choices.

Protease-activated receptor 1 (PAR1) is a G protein-coupled receptor and activated by thrombin.[2] PAR1 can also be activated via plasmin, factor Xa, activated protein C and SFLLRN (synthetic peptides that correspond to the first few amino-acids of freshly cleaved N terminus).[3] There are four different PARs: PAR1 and PAR3 are activated by thrombin, PAR2 is activated by tryptase or trypsin, and PAR4 is activated by both trypsin and thrombin.[3]

PAR1 plays an important role in a variety of physiological responses such as angiogenesis, coagulation, inflammation, mitogenesis, vascular remodeling, cell proliferation, placental implantation, cell survival, and repair of injured tissue.[4] PAR1, also an oncogene,[4,5] is thought to be involved in tumoral invasion and metastatic process of some malignant tumors such as melanoma, breast, prostate, colon and pancreatic cancers.[3,4] Nevertheless, the mechanism how PAR1 affects invasion is not known exactly.[3]

* Corresponding author. Tel.: +90 212 414 71 71, +90 544 594 45 88 (GSM); fax: +90 212 414 71 72.
E-mail addresses: r.atabey@hotmail.com (R. Ciftci), faruktas2002@yahoo.com (F. Tas), elifbilgin85@gmail.com (E. Bilgin), drkeskin76@hotmail.com (S. Keskin), dr_ibrahim2000@yahoo.com (I. Yildiz), dduranyildiz@yahoo.com (D. Duranyildiz), pinarsaip@gmail.com (P. Saip), hasankaranlik@yahoo.com (H. Karanlik), sezai@istanbul.edu.tr (S. Vatansever).
Peer review under responsibility of Turkish Society of Medical Oncology
[1] Tel.: +90 212 414 24 34, +90 532 237 95 70 (GSM); fax: +90 212 534 80 78.
[2] Tel.: +90 212 414 24 34, +90 505 740 63 18 (GSM); fax: +90 212 534 80 78.
[3] Tel.: +90 212 414 24 34, +90 532 575 54 00 (GSM); fax: +90 212 534 80 78.
[4] Tel.: +90 212 414 24 34, +90 505 746 51 78 (GSM); fax: +90 212 534 80 78.
[5] Tel.: +90 212 414 24 34, +90 542 415 46 08; fax: +90 212 534 80 78.
[6] Tel.: +90 212 414 24 34, +90 533 247 36 08; fax: +90 212 534 80 78.
[7] Tel.: +90 212 414 24 34, +90 532 402 53 54; fax: +90 212 534 80 78.
[8] Tel.: +90 212 414 24 34, +90 532 201 01 41; fax: +90 212 534 80 78.

http://dx.doi.org/10.1016/j.jons.2016.04.003

Table 1
Patient characteristics

Variables	*n* (%)
No. of patients	96 (100)
Age, years	
≤49/≥50	51 (53)/45 (47)
Grade	
1/2/3/unknown	4 (4)/26 (27)/24 (25)/42 (44)
ER	
−/+/unknown	27 (28)/68 (71)/1 (1)
PR	
−/+/unknown	32 (33)/63 (66)/1 (1)
HER2	
−/+/unknown	64 (67)/31 (32)/1 (1)
Classification	
Luminal/HER2 +/Triple (−)/Triple (+)	56 (58)/18 (19)/8 (8)/13 (14)
T status[a]	
1/2/3/4/unknown	18 (30)/21 (36)/3 (5)/3 (5)/14 (24)
N status[a]	
−/+/unknown	23 (39)/23 (39)/13 (22)
M status	
−/+	59 (61)/37 (39)
Serum LDH level (450 U/L)	
Normal/Elevated	69 (73)/26 (27)
Serum CA15−3 level (35 U/mL)	
Normal/Elevated	68 (72)/26 (28)
Response to chemotherapy[b]	
Yes/No/unknown	46 (78)/10 (17)/3 (5)

[a] In 59 non-metastatic disease.
[b] In 37 metastatic and 22 locally advanced disease.

PAR1 expression is correlated directly with tumoral invasiveness in cancer cell lines,[6–8] and increased by up to 10-fold in invasive BC compared with normal tissue.[9] In BC cell lines, there is no PAR1 expression on non-metastatic cells or normal tissue, suggesting that PAR1 expression is associated with metastatic potential of BC cell lines.[10] A study including 136 patients with BC showed that PAR1 overexpression in BC tissue is significantly correlated with high grade, advanced stage and poor prognosis.[3] In another study, stromal PAR1 expression in BC tissue specimens was correlated with aggressive tumor behavior.[11] In contrast to mentioned studies, Kamath et al demonstrated that PAR1 signaling inhibits migration and invasion of BC cells.[12] On the other hand, blocking PAR1 had apoptotic effect on tumor and could inhibit lung metastasis in mice with BC.[13,14]

Taken together, data about the relationship between PAR1 and BC consists of only studies including tissue specimens, cell lines or animal studies. Nevertheless, clinical significance of serum PAR1 levels in BC patients is not known and measuring PAR1 level in serum is more feasible than evaluating it in tissue. We performed this study to determine whether serum PAR1 level of BC patients is different from healthy controls, or it has predictive or prognostic value.

2. Patients and methods

2.1. Patients and treatment

This study enrolled 96 consecutive female patients admitted to Institute of Oncology, Istanbul University from March 2010 to January 2013. All patients were diagnosed as invasive breast cancer (BC) pathologically and had not received chemotherapy (CT), hormonotherapy, targeted therapy or radiotherapy within the last 6 months. The TNM staging was determined according to the American Joint Committee on Cancer (AJCC) staging system (7th edition). The pretreatment evaluation included detailed clinical history and physical examination with a series of biochemistry tests, including serum lactate dehydrogenase (LDH) and CA15-3 levels. Blood samples of patients for serum PAR1 measurement was obtained before any type of treatment.

All patients were treated with standard treatment modalities according to NCCN guidelines. Patients received anthracycline- and/or taxane-based CT or single agent capecitabine, trastuzumab (if HER2 positive) with/without radiotherapy depending on the stage of disease. Follow-up programs included clinical, laboratory, and radiological assessments performed at 8-week intervals during CT. Response to treatment was evaluated in patients who had metastatic disease or receiving neoadjuvant CT according to clinical examination and the revised RECIST criteria version 1.1. Those with complete or partial responses were considered as CT responsive. Nodal status was recorded for only non-metastatic patients. Patients were also categorized according to molecular subtypes as: luminal (ER positive, HER2 negative), triple positive (ER, PR and

Table 2
The values of serum PAR-1 levels in patients with breast cancer and healthy controls

	Patients ($n = 96$)	Controls ($n = 30$)	p
Assay	Mean (±SE)	Mean (±SE)	
PAR-1 (ng/ml)	3.07 (4.43)	2.82 (3.63)	**0.011**

Table 3
Comparisons of serum PAR1 levels according to various clinical/laboratory parameters

Variables	PAR-1 (ng/ml) *p* value Mean (±SE)
Age, years	0.72
Young (−49)	3.37 (4.46)
Older (50+)	2.74 (4.27)
Grade	0.055
I (Good)	8.15 (5.33)
II + III (Medium-Poor)	2.65 (4.11)
ER	0.82
Negative	2.85 (4.52)
Positive	3.2 (4.45)
PR	0.71
Negative	3.19 (4.83)
Positive	3.05 (4.29)
HER2	0.25
Negative	3.4 (4.45)
Positive	2.49 (4.46)
Classification	0.49
Luminal	3.42 (4.5)
Others	2.56 (4.34)
Classification	0.43
Triple positive	2.03 (4.19)
Others	3.21 (4.47)
Classification	0.23
Triple negative	3.08 (3.99)
Others	3.07 (4.49)
Classification	0.39
HER2 enriched	2.76 (4.82)
Others	3.15 (4.36)
Axillary lymph node	0.81
N−	2.28 (3.54)
N+	3.01 (4.5)
Metastasis	0.86
M−	2.61 (3.84)
M+	3.81 (5.22)
Tumor size	0.62
T1	3.69 (4.84)
T2-4	2.2 (3.46)
LDH	0.35
Normal	3.41 (4.66)
High	2.26 (3.79)
CA15.3	0.41
Normal	3.07 (4.5)
High	3.21 (4.5)
Chemotherapy response	0.052
No	2.32 (4.24)
Yes	3.83 (4.87)

Table 4
Univariate analysis of survival

Variables	2-year survival rates (±SE)	p
All patients	80 ± 5	
Age, years		0.118
Younger (−49)	87 ± 6	
Older (50+)	72 ± 8	
Grade		0.19
I + II (Good + Medium)	44 ± 10	
III (Poor)	58 ± 10	
ER		**0.007**
Negative	77 ± 9	
Positive	83 ± 6	
PR		**0.004**
Negative	73 ± 9	
Positive	86 ± 5	
HER2		0.55
Negative	77 ± 7	
Positive	88 ± 6	
Classification		0.41
Luminal	82 ± 6	
Others	77 ± 8	
Classification		0.2
Triple positive	75 ± 16	
Others	80 ± 5	
Classification		0.073
Triple negative	48 ± 21	
Others	83 ± 5	
Classification		0.105
HER2 enriched	94 ± 6	
Others	75 ± 6	
Tumor size		0.398
T1	100 ± 0	
T2-4	94 ± 5	
Node status		0.35
Negative	100 ± 0	
Positive	94 ± 6	
Metastasis status		**<0.001**
Negative	97 ± 3	
Positive	51 ± 10	
Serum LDH level		**0.042**
Normal	83 ± 5	
High	68 ± 11	
Serum CA 15−3 level		0.083
Normal	84 ± 5	
High	69 ± 11	
Response to chemotherapy		**<0.001**
Yes	84 ± 7	
No	18 ± 13	
Serum PAR-1 level		0.73
High (>mean)	81 ± 10	
Low (<mean)	79 ± 6	

HER2 positive), triple negative (ER, PR and HER2 negative), and HER2 enriched (ER and PR negative, HER2 positive).

For comparison of serum PAR1 assays, 30 age-matched healthy female controls were included in the analysis. Institutional review board approval was obtained before the commencement of the study.

2.2. Measurement of serum PAR1 levels

Blood samples were obtained from patients with BC and healthy controls by venipuncture and clotted at room temperature. The sera were collected following centrifugation and frozen immediately at −20 °C until analysis.

Serum PAR1 (USCN, Wuhan, PRC) levels were determined by the solid-phase sandwich ELISA method. The microtiter plate has been pre-coated with an antibody specific to PAR1. Standards and samples were added to the wells with a biotin-conjugated antibody specific to PAR1. Next, Avidin conjugated to Horseradish Peroxidase (HRP) was added to each well and incubated. After TMB substrate solution was added, the wells that contain PAR1, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a change in color. The enzyme−substrate reaction was terminated by the addition of sulphuric acid solution and the color change was measured spectrophotometrically (Rayto, RT-1904C Chemistry Analyzer, Atlanta GA, USA) at a wavelength of 450 nm. The concentration of PAR1 in the samples was then determined by comparing the O.D. of the samples to the standard curve.

2.3. Statistical analysis

Comparisons of serum PAR1 levels in BC patients and controls, in addition to patient subgroups according to various clinical/pathological/laboratory parameters were carried out using the Mann−Whitney *U*-test since serum PAR1 had non-normally distribution in all groups. Continuous variables were presented as mean ± standard deviation (SD). Overall survival (OS) was calculated from the date of venous blood sampling for PAR1 analysis to death resulting from any cause or to last contact with the patient or any family member. The Kaplan−Meier method was used for the estimation of the OS of patients and differences in survivals were assessed using the log-rank test. We also assessed whether serum PAR1 has an independent prognostic effect by performing a multivariate analysis with Cox's regression analysis. While investigating the relationships between serum PAR1 level and other parameters, the correlation coefficients and their significance were calculated using Spearman test. A p value less than 0.05 was accepted as statistically significant. Statistical analysis was carried out using SPSS 18.0 software (SPSS Inc., Chicago, Illinois, USA).

3. Results

The baseline histopathological and the demographic characteristics of the patients are listed in Table 1. The median age at diagnosis was 48 years, with a minimum and maximum values of 29−80 years.

The serum PAR1 levels showed a statistically significant difference between the BC patients and the control groups (Table 2). Serum PAR1 level was significantly higher in the BC group than controls (mean level of 3.07 vs 2.82 ng/ml, $p = 0.011$).

PAR1 levels tended to be higher among CT responders ($p = 0.052$) and grade I disease ($p = 0.055$) than others (Table 3). There were no significant correlations between serum PAR1 levels and any other type of clinic-pathological and laboratory variables.

Median follow-up period was 22 (±9.85) months. The 2-year survival rate for all the patients were 80% (±5). As expected, negative ER and PR status, high LDH level, presence of metastasis, absence of CT response were unfavorable factors significantly influencing OS in univariate analysis (Table 4).

Serum PAR1 level did not have a significant effect on OS in univariate analysis (Table 4 and Fig. 1). The 2-year survival rates for patients with high (>mean) and low (<mean) serum PAR1 levels were 81% (±10) vs. 79% (±6), respectively ($p = 0.73$) (Table 4). Multivariate analysis including PAR1 level, ER status, presence of metastasis, CT response and LDH level revealed that only metastatic status has an independent significant impact on survival (HR: 8.81, 95% CI: 1.08−71.59, $p = 0.04$) (Table 5). Serum PAR1 level had no statistically significant effect on survival in multivariate analysis (HR: 0.75, 95% CI: 0.2−2.82, $p = 0.67$).

4. Discussion

It has been established that PAR1 is overexpressed in metastatic cell lines and different carcinoma biopsy specimens, while its expression is minimal or absent in normal breast tissue and non-

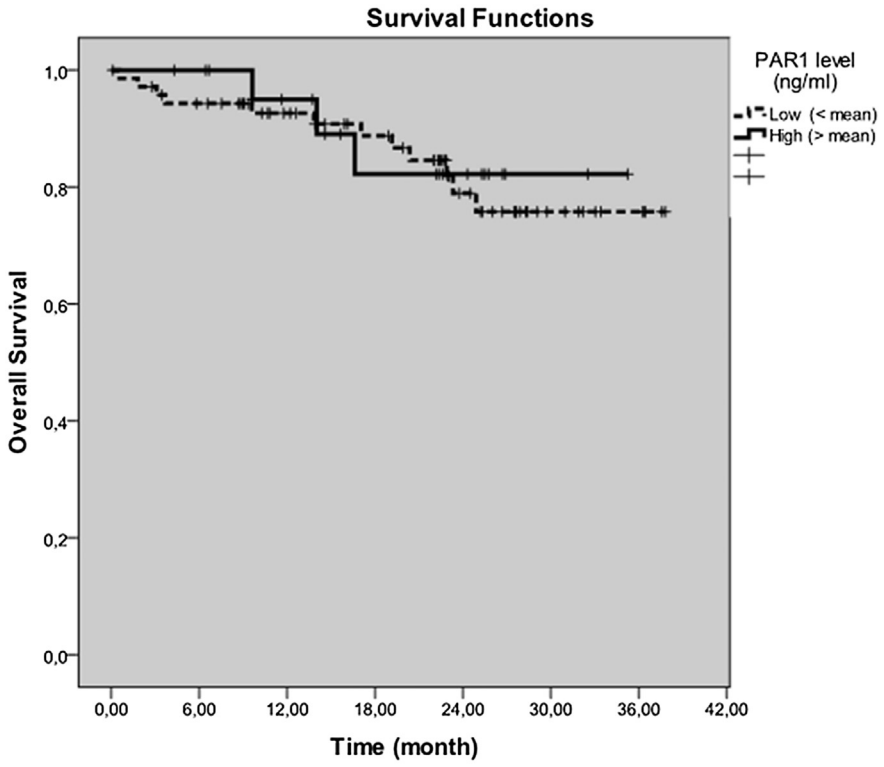

Fig. 1. Survival curves of breast cancer patients according to serum PAR1 levels ($p = 0.73$)

invasive lesions.[7,10] PAR1 overexpressing cancer cells are more invasive in vitro and become much more invasive by a PAR1 activating ligand.[7,10,15]

In many experimental models, it has been demonstrated that PAR1 plays an important role in invasive and metastatic process of many tumors such as BC,[4,10] pancreatic carcinoma,[16] melanoma,[17] bladder, ovarian, colon and prostate cancers.[4] In addition, inhibition of PAR1 results in inhibition of tumor invasion and migration in vitro.[7,18] Similarly, activation of PAR1 overexpressing cells by thrombin or specific PAR1 activating peptide SFLLRNP increases tumor cell survival in vitro and in vivo in mice, whereas withdrawal of PAR1 leads to selective apoptosis in immature blood vessels and tumor cells[13] and decrease invasiveness of BC cells.[10,19] In contrast to all these studies, Kamath et al demonstrated that PAR1 signaling has inhibitory effect on migration and invasion of BC cells.[12]

In a study, tissue specimens of 224 BC patients with a majority of histological grade I or II were assessed retrospectively.[11] Stromal PAR1 expression was significantly higher in hormone-negative BC tissue compared with hormon-positive ones, suggesting that PAR1 is correlated with aggressive tumor behavior. On the other hand, PAR1 expression in tumor cells did not significantly correlate with any of the other clinic-pathological parameters including histological grade, nodal status, molecular subtypes, expression of ER, PR or HER2.

Table 5
Multivariate analysis of overall survival

Variables	HR	95% CI	p
Chemotherapy response: yes *v* no	0.39	0.13–1.18	0.09
ER status: positive *v* negative	0.84	0.25–2.8	0.77
Metastasis status: positive *v* negative	8.81	1.08–71.59	**0.04**
Serum PAR1 level: high *v* low	0.75	0.2–2.82	0.67
LDH level: high *v* normal	1.38	0.48–3.99	0.54

HR: Hazard ratio, CI: Confidence interval.

The mechanism how PAR1 involves in tumor invasion has not been completely understood. Impaired degradation of activated PAR1 which results in sustained PAR1 signaling is specific for only invasive BC cells and may be an explanation.[2] The vascular endothelial growth factor (VEGF),[4] Akt pathway[13] and matrix metalloproteases (MMPs), especially MMP-1[20] appear to be important co-operators of PAR1. PAR1 induces both angiogenesis and tumor growth by increasing VEGF expression.[4,13] MMP-1 is mainly produced by stromal cells rather than tumoral cells and stromal MMP-1 has been shown to cleave and activate PAR1.[20] MMP-1 is upregulated in BC and also in gastric and esophageal cancers.[21–23] PAR1 activation can also initiate Akt signaling.[13] Some studies suggest that Akt signaling induces tumor progression and angiogenesis,[14,24,25] while other studies[26,27] suggest that activation of Akt pathway can prevent metastatic behavior of BC cells.

The only study researching the survival effect of PAR1 expression in the cancer tissues of BC patients has been reported by Hernandez et al.[3] Among 136 patients, PAR1 expression was observed in only high grade patients ($n = 50$). Majority of PAR1 expressing patients (58%) had PAR1 overexpression. During the median 95 months of follow-up, metastasis developed in only PAR1 overexpressing patients and 62% of them died. PAR1 overexpression was significantly correlated with stage, HER1 and ER expression, increased mortality and metastasis development.

So far, unfortunately, PAR1 level has not been studied in serum of BC patients. This is the first study evaluating the serum PAR1 level in BC patients. In the current study, we demonstrated that serum PAR1 level is significantly higher in BC patients than healthy controls and may have predictive role for CT response. However, serum PAR1 level did not have prognostic value or significant correlations with the stage of the disease or other known disease-related parameters. Our results appear to be not consistent with majority of previous studies demonstrating presence of PAR1 overexpression and its association with tumoral invasiveness in BC

tissue or cell lines.[2–4,10,11,13] Nevertheless, comparing a study measuring serum PAR1 level with results of experimental (in vivo or in vitro) or tissue studies is difficult to interpret. There is no data in literature about the relationship between serum PAR1 level and its expression level in cancer tissue of BC patients. Serum PAR1 level, of course, may not reflect its tissue level or functional status properly. However, measuring serum PAR1 level is more feasible than assessing its expression in tissue.

In conclusion, although serum PAR1 level is elevated in BC patients and has a probable predictive value for CT response, it does not have a prognostic value. The short follow-up time could be considered as a significant limitation of our study and might have masked prognostic value of serum PAR1 level. However, to our knowledge this is the first study evaluating the clinical significance of serum PAR1 level in BC patients. Further prospective studies with longer follow-up are needed investigating both the correlation of PAR1 overexpression in BC tissue and serum PAR1 levels and whether serum PAR1 level changes after treatment or it has a prognostic value.

Conflict of interest

The authors do not have any conflict of interest to declare.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127:2893–2917.
2. Booden MA, Eckert LB, Der CJ, Trejo J. Persistent signaling by dysregulated thrombin receptor trafficking promotes breast carcinoma cell invasion. *Mol Cell Biol*. 2004;24:1990–1999.
3. Hernández NA, Correa E, Avila EP, Vela TA, Pérez VM. PAR1 is selectively over expressed in high grade breast cancer patients: A cohort study. *J Transl Med*. 2009;7.
4. Yin YJ, Salah Z, Grisaru-Granovsky S, et al. Human protease-activated receptor 1 expression in malignant epithelia: A role in invasiveness. *Arterioscler Thromb Vasc Biol*. 2003;23:940–944.
5. Martin CB, Mahon GM, Klinger MB, et al. The thrombin receptor, PAR-1, causes transformation by activation of Rho-mediated signaling pathways. *Oncogene*. 2001;20:1953–1963.
6. Boire A, Covic L, Agarwal A, Jacques S, Sherifi S, Kuliopulos A. PAR1 is a matrix metalloprotease receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell*. 2005;120:303–313.
7. Even-Ram SC, Maoz M, Pokroy E, et al. Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the alpha vbeta 5 integrin. *J Biol Chem*. 2001;276:10952–10962.
8. D'Ándrea MR, Derian CK, Santulli RJ, Andrade-Gordon P. Differential expression of protease activated receptors-1 and -2 in stromal fibroblasts of normal, benign and malignant human tissues. *Am J Pathol*. 2001;158:2031–2041.
9. Korkola JE, DeVries S, Fridlyand J, et al. Differentiation of lobular versus ductal breast carcinomas by expression microarray analysis. *Cancer Res*. 2003;63:7167–7175.
10. Even-Ram S, Uziely B, Cohen P, et al. Thrombin receptor overexpression in malignant and physiological invasion processes. *Nat Med*. 1998;4:909–914.
11. Tiburcio M, Costa SM, Fatima Duarte M, Schmitt FC, Longatto Filho A. Characterization of PAR1 and FGFR1 expression in invasive breast carcinomas: prognostic significance. *Oncol Lett*. 2012;4:647–657.
12. Kamath L, Meydani A, Foss F, Kuliopulus A. Signaling from protease-activated receptors-1 inhibits migration and invasion of breast cancer cells. *Cancer Res*. 2001;61:5933–5940.
13. Salah Z, Maoz M, Pokroy E, Lotem M, Bar-Shavit R, Uziely B. Protease-activated receptor-1 (hPar1), a survival factor eliciting tumor progression. *Mol Cancer Res*. 2007;5:229–240.
14. Yang E, Boire A, Agarwal A, et al. Blockade of PAR1 signaling with cell-penetrating pepducins inhibits Akt survival pathways in breast cancer cells and suppresses tumor survival and metastasis. *Cancer Res*. 2009;69:6223–6231.
15. Henrikson KP, Salazar SL, Fenton II JW, Pentecost BT. Role of thrombin receptor in breast cancer invasiveness. *Br J Cancer*. 1999;79:401–406.
16. Rudroff C, Seibold S, Kaufmann R, et al. Expression of the thrombin receptor PAR-1 correlates with tumour cell differentiation of pancreatic adenocarcinoma in vitro. *Clin Exp Metastasis*. 2002;19:181–189.
17. Tellez C, McCarty M, Ruiz M, Bar-Eli M. Loss of activator protein-2 a results in overexpression of protease-activated receptor-1 and correlates with the malignant phenotype of human melanoma. *J Biol Chem*. 2003;278:46632–46642.
18. Albini A, Iwamoto Y, Kleinman HK, et al. A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Res*. 1987;47:3239–3245.
19. Nierodzik ML, Chen K, Takeshita K, et al. Protease-activated receptor 1(PAR1) is required and rate limiting for thrombin enhanced experimental pulmonary metastases. *Blood*. 1998;92:3694–3700.
20. Nguyen N, Kuliopulos A, Graham RA, Covic L. Tumor -derived Cyr61 (CCN1) promotes stromal matrix metalloproteinase-1 production and protease-activated receptor1-dependent migration of breast cancer cells. *Cancer Res*. 2006;66:2658–2665.
21. Poola I, DeWitty RL, Marshalleck JJ, Bhatnagar R, Abraham J, Leffall LD. Identification of MMP-1 as a putative breast cancer predictive marker by global gene expression analysis. *Nat Med*. 2005;11:4813.
22. Murray GI, Duncan ME, O'Neil P, McKay JA, Melvin WT, Fothergill JE. Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. *J Pathol*. 1998;185:256–261.
23. Migita T, Sato E, Saito K, et al. Differing expression of MMPs-1 and-9 and urokinase receptor between diffuse-and intestinal-type gastric carcinoma. *Int J Cancer*. 1999;84:74–79.
24. Irie HY, Pearline RV, Grueneberg D, et al. Distinct roles of Akt1 and Akt2 in regulating cell migration and epithelial-mesenchymal transition. *J Cell Biol*. 2005;171:1023–1034.
25. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2:489–501.
26. Yoeli Lerner M, Yiu GK, Rabinovitz I, Erhardt P, Jauliac S, Toker A. Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. *Mol Cell*. 2005;20:539–550.
27. Liu H, Radisky DC, Nelson CM, et al. Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. *Proc Natl Acad Sci USA*. 2006;103:4134–4139.