Rho GDP dissociation inhibitor α expression correlates with the outcome of CMF treatment in invasive ductal breast cancer

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Abstract. Rho-GDIα is an inhibitor of Rho-GTPases, which is involved in cancer progression. Little is known about its role in breast cancer progression. There is evidence, that Rho-GDIα may modulate drug resistance of breast cancer cells. To assess the importance of Rho-GDIα as a risk factor in invasive ductal breast cancer, cancer specimens of three groups of patients were analyzed for Rho-GDIα RNA (group 1, N=72 and group 2, N=73) or protein expression (group 3, N=90). In group 1, patients did not receive any adjuvant treatment, whereas, in groups 2 and 3, patients were treated with anti-estrogens and/or with chemotherapeutical drugs. Rho-GDIα RNA levels, measured by RT-PCR from fresh-frozen material, did not correlate with relapse-free survival in Kaplan-Meier analysis, except in a subgroup of CMF-only treated patients. In this subgroup, higher Rho-GDIα RNA levels were significantly associated with more favorable prognosis. Immunohistochemical analysis (group 3) confirmed the link between higher Rho-GDIα expression and better outcome. This was again particularly true for the CMF-only treated patients. Cox regression analysis revealed that high Rho-GDIα protein expression reduced the risk for a relapse by ~3-fold, even if adjusted for grading, tumor size, nodal and estrogen receptor (ER) status. The data suggest that Rho-GDIα is beneficial to patients who received adjuvant chemotherapy. Rho-GDIα is possibly a useful biomarker to predict the response of breast cancer patients to CMF treatment.

Introduction

The family of Rho-GTPases belongs to the Ras-like protein superfamily (1-3). Rho-GTPases play important roles in a number of cellular functions, which include cellular migration and adhesion. By modulating actin dynamics Rho-GTPases are key players in the regulation of filopodia and lamellopodia formation. As a consequence of their migration-promoting activity Rho-GTPases, such as RhoA, Rac1 or Cdc42, contribute to cancer progression (4). Typically for Ras-like proteins, Rho-GTPases are regulated by GTP/GDP and cycle between an active membrane-bound GTP-captured form and an inactive, cytosolic GDP-bound form. Two families of proteins, GEFs (guanine-nucleotide-exchange factors) and GAPs (GTPase-activating proteins), are major regulators of the Rho-GTPase GTP/GDP status. Additionally, unlike Ras-GTPases, Rho-GTPases are also controlled by a family of inhibitors, called Rho-GDIs (Rho GDP dissociation inhibitors). Rho-GDIs stabilize the inactive GDP-bound form of Rho-GTPases and keep it soluble in the cytosol. In addition, these proteins help to shuttle and present certain Rho-GTPases to certain effectors (2). Three members of the Rho-GDI family, Rho-GDIα (Rho-GDI, ARHGDIA), Rho-GDIß (Ly-GDI, D4-GDI, ARHGDIB) and Rho-GDIγ (ARHGDIG), are known. Rho-GDIα is ubiquitously expressed, whereas the expression of Rho-GDIß and Rho-GDIγ is more cell type-restricted. Rho-GDIα and Rho-GDIß are also expressed in epithelial cancer cells (5-7). In bladder and lung cancer, Rho-GDIß suppresses invasion and metastasis (5,6) and, in bladder cancer, high Rho-GDIß levels predict favorable prognosis (8). In breast cancer, Rho-GDIß is regulated by the oncoprotein Ets1 and has a dual function as it promotes the expression of the oncoprotein cyclooxygenase 2 (Cox-2) and, at the same time, inhibits cellular migration (9). Probably due to its dual function Rho-GDIß is not a suitable biomarker for predicting the outcome of breast cancer patients. In contrast to the findings on Rho-GDIß, there are only few studies available on the role of Rho-GDIα in cancer progression and drug resistance. E.g., in colorectal cancer, overexpression of Rho-GDIα has been shown to be correlated with reduced survival (10). Based on data obtained by cell culture experiments Rho-GDIα has also been suggested to contribute to drug resistance (11,12). Therefore, we explored the possibility that Rho-GDIα RNA and protein levels are associated with the outcome of patients suffering from invasive ductal carcinoma. We found that high levels of Rho-GDIα in breast cancer are beneficial to breast cancer patients when treated with chemotherapeutics.

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Materials and methods

Cell lines. MCF-7 and MDA-MB-231 breast cancer were maintained in RPMI medium (Invitrogen) supplemented with 10% fetal calf serum (PAN) in the absence of antibiotics.

Quantitative RT-PCR. After primary surgery, a representative part of tumor was selected by a pathologist and, frozen in liquid nitrogen. Aliquots of tissue were pulverized by using a microdismembrator (Braun, Melsungen, Germany) and kept in liquid nitrogen until RNA isolation. Total RNA was isolated from 20 mg of tissue powder using the RNeasy mini kit (Qiagen, Hilden, Germany) with on-column DNase-I treatment. Quality of the RNA was checked by examining ribosomal RNA bands after agarose gel-electrophoresis. For quantitative PCR analysis, total RNA was transcribed into cDNA using random hexamers and Mo-MLV reverse transcriptase (Invitrogen). The relative RNA level was calculated relative to the RNA level of the house-keeping gene HPRT (hypoxanthine guanine phospho ribosyltransferase) (13). Primers used for the amplification of the Rho-GDIα specific cDNA were as follows, forward primer: 5'-AACCGA GAGATAGTGTCCGGC-3', reverse primer: 5'-TCTTGA CGCCTTTCCTGTACG-3' (MWG Biotech). Primers for the detection of Rho-GDIß are described in Schunke *et al* (9). The cut-off for separating tumors with high Rho-GDIα levels from those with low levels was set at the median level of all 263 measured tumors from the tumor bank in the Department of Chemical Endocrinology, Radboud University Nijmegen Medical Centre (The Netherlands).

Protein extraction and Western blot analyses. Extraction of cytosolic proteins from cultured cells and Western blot analysis were performed as described (14). Extraction of cytosolic proteins from tumor tissue was carried out according to Schunke *et al* (9). To visualize Rho-GDIα or Rho-GDIß protein the protein blot was incubated with a monoclonal mouse anti-Rho-GDI antibody or a rabbit polyclonal anti-D4-GDI antibody (BD Biosciences) at a dilution of 1:1000 or 1:5000, respectively. To check for equal protein loading the blot was reprobed with an ERK1/2-specific antibody, diluted 1:1000, from Cell Signaling (15). Anti-mouse or anti-rabbit secondary antibodies conjugated with horseradish peroxidase were purchased from Cell Signaling. Chemiluminescent signals were detected by using ECL-Plus and Hyperfilm ECL (GE-Amersham).

Immunohistochemistry. Immunohistochemical staining of paraffinized breast cancer samples were carried out as described (9). Briefly, sections were deparaffinized by subsequent treatments with xylene (2x10 min), 100% ethanol (2x5 min), 96% ethanol (2x5 min), 70% ethanol (2x5 min). To block endogenous peroxidases, sections were treated with hydrogen peroxidase for 15 min. De-masking was performed in citrate buffer (29.4 g trisodium citrate dihydrate/l, pH 6.0) at 70°C for 45 min. After blocking in a blocking solution (Zytomed, Berlin, Germany) for 5 min, slides were incubated with the anti-Rho-GDIα antibody or anti-Rho-GDIß (both diluted 1:500) at 4°C overnight. For detection of the primary antibody a biotinylated secondary antibody/streptavidin horse peroxidase conjugate-based assay (Zytomed, HRP060) was used by following the manufacturer's instructions. The antibody complexes were visualized by using an AEC substrate kit (Zytomed). After incubation at room temperature for 20 min, the reaction was stopped by rinsing the slides with water. Nuclei were stained by hematoxylin. To quantify the immune reaction an immunoreactive score (IRS) was determined. The IRS was calculated by multiplying staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong) by the percentage of stained tumor cells (0, no cells stained; 1 <10% of cells stained; 2, 11-50% of cells stained; 3, 51-80% of cells stained; 4 >81% of cells stained). By setting the cut-off for high expression of Rho-GDIα at an IRS of 8 the 90 invasive breast cancer specimen were equally divided into two groups, one with low and one with high expressing cancer specimens.

Breast cancer biopsies. Breast cancer specimens from 263 breast cancer patients (Nijmegen, The Netherlands) with unilateral operable breast cancer were analyzed. These patients were first diagnosed with breast cancer between 1987-1997. After resection of the tumor patients were systemically treated with anti-estrogen and/or chemotherapeutics or left untreated. Coded tumor tissues were used in accordance with the Code of Conduct of the Federation of Medical Scientific Societies in The Netherlands. This study adhered to all relevant institutional and national guidelines. Of the 263 breast cancer specimen 145 were invasive ductal cancers that were used for the analysis. Of 145 patients with invasive ductal cancer 72 were not treated and 73 received adjuvant endocrine and/or chemotherapeutical treatment. Paraffin sections of invasive breast cancer specimen from 90 patients (Halle, Germany) were analyzed for the expression of Rho-GDIα protein by using immunohistochemistry. Patients of this cohort were first diagnosed with cancer in 1999 or 2000 and all received adjuvant treatment. This study was approved by the Institutional Review Board.

Statistical methods. Univariate survival analysis was performed by using the Kaplan-Meier method. The significance of differences in survival curves were evaluated by the log-rank test. Cox regression analysis was carried out to calculate the hazard ratios for risk factors. All statistical analyses were done with SPSS 17.0 software (SPSS Inc.). $P<0.05$ was considered significant.

Results

Clinicopathological factors. For the analysis of Rho-GDIα expression in invasive ductal breast cancer we used a total of 235 breast cancer specimen. Of these, 145 were fresh-frozen biospies from a tumor bank in the Department of Chemical Endocrinology at the Radboud University in Nijmegen Medical Centre (The Netherlands) and 90 were formalin-fixed paraffin-embedded biopsies from a tumor bank in the Institute for Pathology at the University of Halle/Saale (Germany). Of the 145 fresh-frozen biopsies, which were all used for the measurement of Rho-GDIα-RNA levels, 72 originated from patients that did not receive any adjuvant treatment after the cancer has been surgically removed and 73 were from patients that were systemically treated with

Table I. Clinicopathological data of the patients analyzed.

Variable	RhoGDIα-RNA		RhoGDIα-protein
	Untreated in %	Treated in %	Treated in %
Tumor type			
Invasive, ductal	100 (N=72)	100 (N=73)	100 (N=90)
Type of adjuvant treatment			
Endocine	n.a.	64	31
CMF only	n.a.	16	22
Anthracycline only	n.a.	6.8	3.3
Endocrine + chemo	n.a.	11	40
Others	n.a.	1.4	3.3
Age (years)			
<50	24	26	31
≥50	76	74	69
Menopausal status			
Premenopausal	23	28	38
Postmenopausal	78	72	62
Nodal status			
Negative	78	6	64
Positive	22	94	36
Tumor size			
pT1	29	18	44
pT2	61	60	46
pT3/4	10	22	10
Histological grade			
I	5	6	3
II	33	41	49
III	32	36	48
ER			
Negative	41	31	42
Positive	59	69	58
PgR			
Negative	43	37	57
Positive	57	63	43

n.a., not applicable.

anti-estrogens and/or chemotherapeutical drugs, such as anthracyclines and/or a combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) (Table I). Most of the clinicopathological parameters were similar between the 72 untreated and the 73 treated patients. One exception was the nodal status. The majority of untreated patients (78%) had no nodal involvement, whereas almost all (94%) of the group of treated patients showed metastasis in the axillary nodes. The 90 biopsies that were formalin-fixed/paraffin-embedded and used for immunochemical determination of the Rho-GDIα protein status were excised from patients that had all received adjuvant treatment. The clinicopathological parameters of this group of patients resemble those of the group of the 72 untreated patients.

The effect of Rho-GDIα RNA overexpression on clinical outcome is different in treated vs. untreated breast cancer patients. We studied the effect of Rho-GDIα RNA levels on disease-free survival. Irrespective of whether patients received or did not receive adjuvant treatment, no significant association between Rho-GDIα RNA levels and disease-free survival could be observed (compare Figs. 1A and 2A). However, a trend was visible. It seems that untreated patients may potentially benefit from lower Rho-GDIα RNA levels,

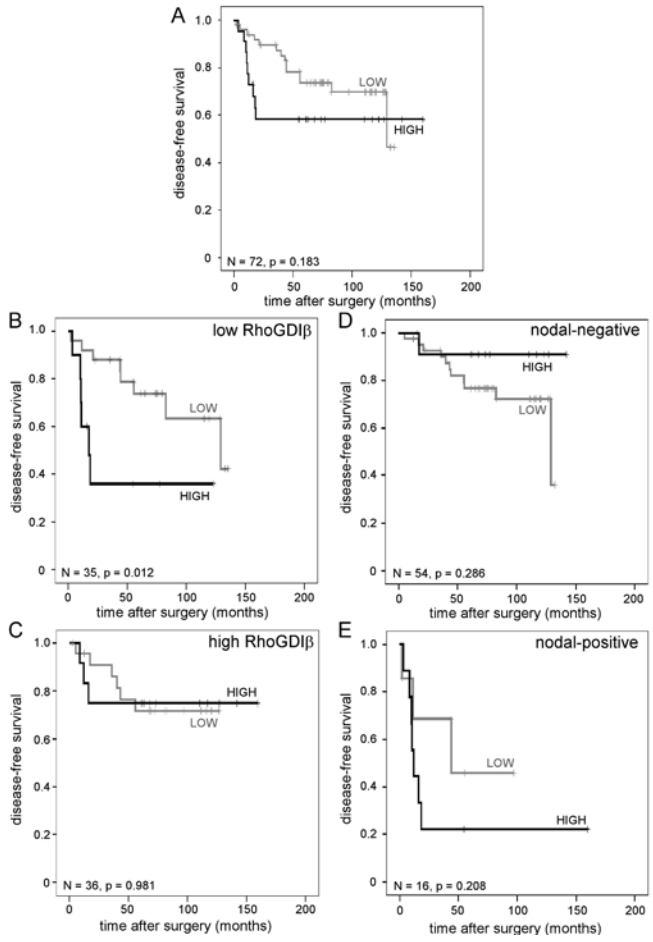

Figure 1. Effect of Rho-GDIα RNA levels on prognosis of untreated patients with invasive ductal breast cancer. Kaplan-Meier survival curves for disease-free survival are shown for high and low Rho-GDIα RNA levels under different conditions: no stratification (A), at low (B) or high (C) tumoral Rho-GDIß RNA levels, at negative (D) or positive (E) nodal status. P-values resulting from the log-rank test are indicated.

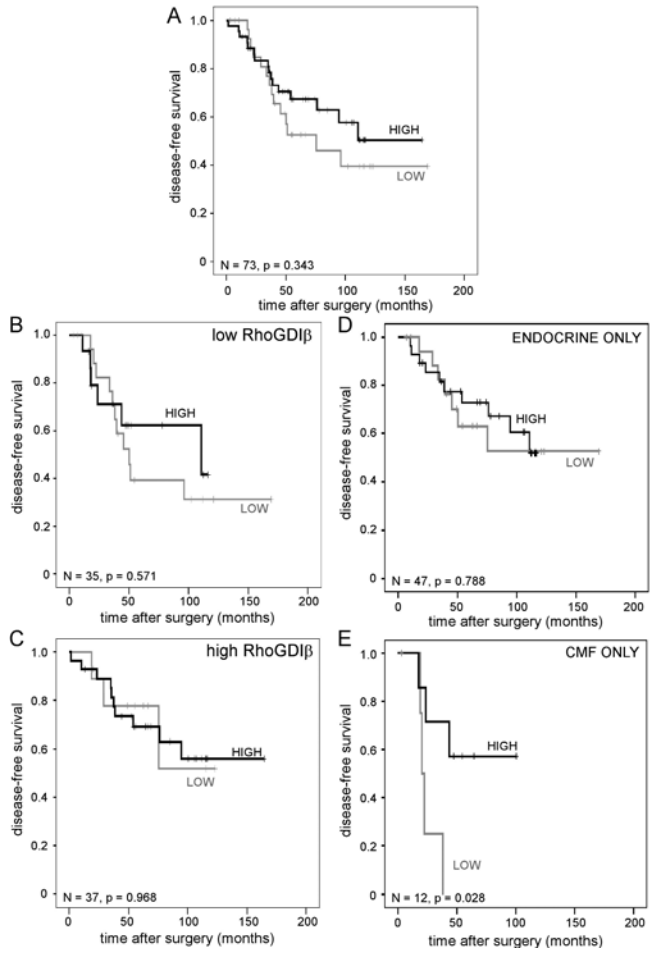

Figure 2. Effect of Rho-GDIα RNA levels on prognosis of treated patients with invasive ductal breast cancer. Effect of Rho-GDIα RNA levels on disease-free survival as analyzed by the Kaplan-Meier method, when the analysis included all treated patients (A) or a subgroup of patients who either showed low (B) or high (C) tumoral expression of Rho-GDIß RNA or who received only endocrine (D) or only CMF (E) treatment. P-values resulting from the log-rank test are indicated.

whereas treated patients may have a better outcome at higher Rho-GDIα RNA levels. To explore the possibility that Rho-GDIß, a close relative of Rho-GDIα, may influence the effect of Rho-GDIα on outcome, we stratified for Rho-GDIß. In the group of treated patients, Rho-GDIß had no significant impact on the effect of Rho-GDIα on the patients' outcome (Fig. 2B and C). In contrast, in the group of untreated patients, the adverse effect of Rho-GDIα on prognosis was significant (p=0.012, log-rank test) in the absence of Rho-GDIß (Fig. 1B), whereas it was completely abolished in the presence of Rho-GDIß (Fig. 1C). These data suggest that, under certain conditions, Rho-GDIß may interfere with effect of Rho-GDIα on patient outcome. In addition to Rho-GDIß, we also stratified for nodal status. Untreated patients seemed to benefit from high Rho-GDIα RNA levels, when the nodes were tumor-free (Fig. 1D). In contrast, when nodes were tumor-positive, high Rho-GDIα levels seemed to be associated with a less favorable outcome (Fig. 1E). It should be noted, however, that, in both cases, the differences were not statistically significant. Such analyses could not be performed for the group of treated patients, as only 4 (6%) out of 73 patients of this group showed no nodal involvement (Table I). We next analyzed the effect of the type of treatment on the correlation between Rho-GDIα RNA expression and prognosis. When patients were treated with anti-estrogens alone, no impact of Rho-GDIα RNA expression on clinical outcome could be observed (Fig. 2D). However, when patients received adjuvant treatment with CMF alone (N=12), higher levels of Rho-GDIα RNA seemed to be beneficial for the patients (Fig. 2E). This correlation was statistically significant (p=0.028, log-rank test). Since all CMF-only treated patients were nodal-positive, they could be directly compared to the nodal-positive untreated patients (compare Fig. 1E with 2E). Apparently, CMF treatment reverses the effect of Rho-GDIα RNA overexpression on the outcome of breast cancer patients. These data suggest that Rho-GDIα RNA overexpression is not *per se* beneficial to breast cancer patients and may even be detrimental to untreated patients (Fig. 1E). Rho-GDIα may sensitize invasive breast cancer to treatment with CMF.

Immunohistochemical studies confirmed data obtained by Rho-GDIα-specific RT-PCR. Two major conclusions could

Table II. Relapse rates and hazard ratios for risk factors of relapse in treated patients with invasive ductal breast cancer.

Risk factor	No. of cases	Cases of high Rho-GDIα protein levels (%)	Correlation P-value	No. of relapses (%)	Hazard ratio (95% CI)	P-value	Hazard ratio adjusted for the other risk factors (95% CI)	P-value
Rho-GDIα protein								
Low	44			15 (34.1)				
High	46			5 (10.9)	0.27 (0.10-0.76)	0.012	0.31 (0.11-0.88)	0.029
Nodal status								
Negative	58	31 (53.4)		11 (19.0)				
Positive	32	15 (46.9)	0.550	9 (28.1)	1.59 (0.66-3.85)	0.303	1.13 (0.42-3.08)	0.808
Grading								
G1/G2	47	28 (59.6)		7 (14.9)				
G3	43	18 (41.9)	0.093	13 (22.2)	2.18 (0.87-5.48)	0.097	0.71 (0.24-2.09)	0.529
Tumor size								
T1	40	22 (55.0)		6 (15.0)				
T2/T3	50	24 (48.0)	0.509	14 (28.0)	2.05 (0.79-5.35)	0.141	1.90 (0.63-5.71)	0.255
ER								
Negative	37	15 (40.5)		16 (43.2)				
Positive	52	31 (59.6)	0.076	4 (7.7)	0.12 (0.04-0.38)	<0.001	0.13 (0.42-3.08)	0.001

[a]Grading, tumor size, nodal and ER status were compared with Rho-GDIα expression. P-values were calculated by cross table analysis using the χ^2 test.

Figure 3. Western blot and immunohistochemical analyses. (A) Western blot analysis of cytosolic extracts from MCF-7 breast cancer cells using a Rho-GDIα-specific antibody. (B) Western blot analyses of cytosolic extracts from primary breast cancer biopsies and from MDA-MB-231 breast cancer cells using antibodies against Rho-GDIα, Rho-GDIß and ERK1/2. (C and D) Immunohistochemical analysis of formalin-fixed, paraffin-embedded invasive ductal breast cancer specimens showing high (C) or low (D) reactivity to the Rho-GDIα antibody.

be drawn from the Rho-GDIα-specific RT-PCR analyses of invasive breast cancer specimens. Correlations of Rho-GDIα overexpression with clinical outcome depends on (i) nodal status and (ii) the type of adjuvant treatment. In an attempt to confirm these data we determined the Rho-GDIα protein level in 90 invasive ductal carcinoma specimens from patients that all received adjuvant treatment and compared them with disease-free survival. Protein measurement was achieved by immunohistochemistry by analyzing the reactivity to a Rho-GDIα-specific antibody. The specificity of this antibody was studied by Western blot analysis by using cytosolic extracts from MCF-7 breast cancer cells. A single band corresponding to a protein of a molecular weight of approximately 27 kDa was detected (Fig. 3A). A similar apparent molecular weight of the 204 amino acid-containing Rho-GDIα protein has been reported by others (12). To show that Rho-GDIα is also expressed in breast cancer tissue, cytosolic proteins from six primary breast cancer biopsies were prepared and analyzed by Western blot analysis. The data show that Rho-GDIα expression varies between biopsies (Fig. 3B). Interestingly, the Rho-GDIα expression pattern is different from that of the closely related protein Rho-GDIß in some tumor samples. While Rho-GDIα and Rho-GDIß are both highly expressed in tumor sample no. 2 and weakly expressed in tumor samples no. 1, 4 and 5, expression is different in samples no. 3 and 6, where either Rho-GDIα or Rho-GDIß expression was more pronounced. Immunohistochemical

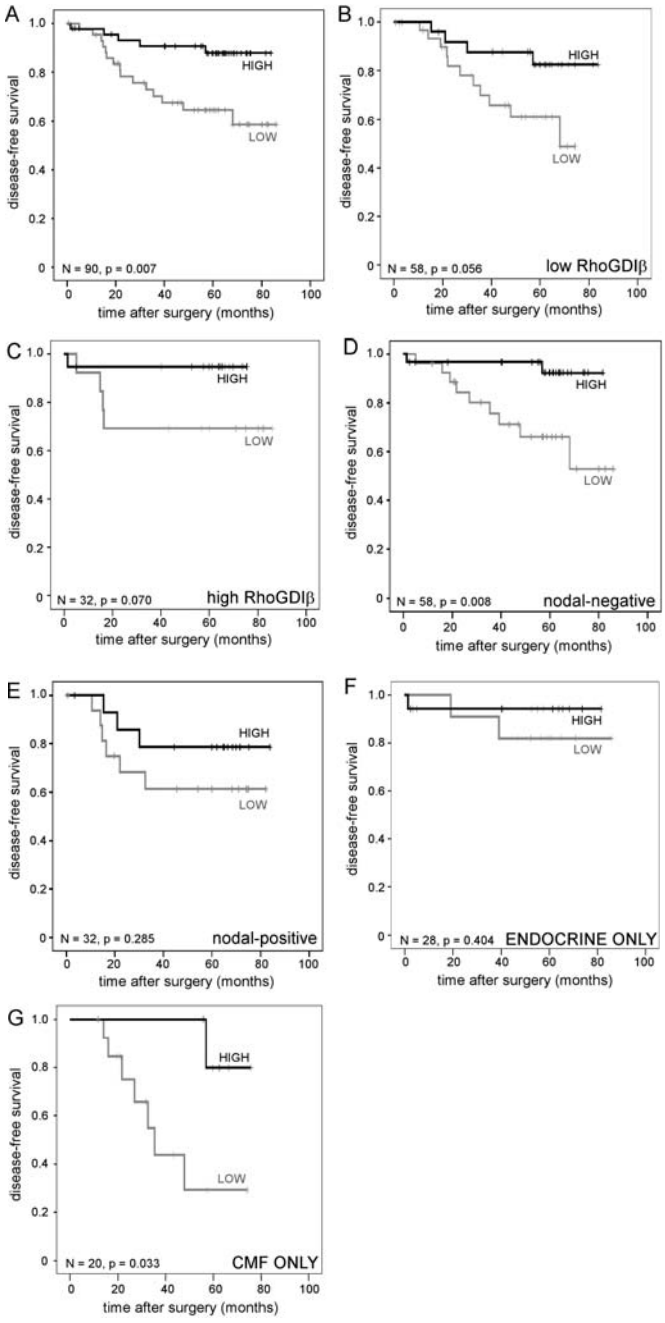

Figure 4. Increased immune reactivity to the Rho-GDIα-specific antibody is correlated with better clinical outcome of patients with invasive ductal breast cancer. Kaplan-Meier survival curves for disease-free survival are shown for high and low Rho-GDIα protein levels. All patients received adjuvant treatment. Subgroup analyses were also performed for Rho-GDIß protein expression (B and C), nodal status (D and E) and type of treatment (F and G). P-values resulting from the log-rank test are indicated.

staining of invasive ductal breast cancers using the Rho-GDIα-specific antibody also revealed strong differences in antibody reactivity between tumor samples (Fig. 3C and D). By using the IRS scoring system as described under Materials and methods, breast cancer specimens were equally divided in low expressing and high expressing cancers. For each group, Kaplan-Meier survival analyses were performed. The data revealed that high expression of Rho-GDIα protein significantly correlated with good prognosis (p=0.007, Fig. 4A). The same tendencies were found in subgroups of patients with high or low tumoral expression of Rho-GDIß protein (Fig. 4B and C). Hence, Rho-GDIß has no impact on the effect of Rho-GDIα on the outcome of treated patients, which is in agreement with the findings obtained by the RNA analyses (Fig. 2B and C). However, when subgroup analyses were done for nodal-positive and nodal-negative patients it was found that only the nodal-negative patients (N=58) highly profited from Rho-GDIα protein overexpression (p=0.008, Fig. 4D). In contrast, the outcome of nodal-positive patients (N=32) was independent of the Rho-GDIα protein expression (Fig. 4C) which is in agreement with the results obtained by Rho-GDIα RNA expression analysis of a similar group of patients (adjuvant treatment, 94% nodal-positive) (Fig. 2A). When patients were subgrouped by type of treatment we found that patients treated with CMF alone had a significantly better prognosis when the cancer produced high levels of Rho-GDIα protein (p=0.033, Fig. 4G). In contrast, patients who received anti-estrogen only did not benefit from higher Rho-GDIα protein expression (Fig. 4F). These data again are in agreement with the results obtained with the Rho-GDIα RNA analysis (compare Fig. 4F with 2D and Fig. 4G with 2E). In particular, the Rho-GDIα protein analysis confirmed that CMF-treated breast cancer patients have a better prognosis if Rho-GDIα expression is high. This supports the hypothesis that Rho-GDIα may sensitize patients to CMF. The data also confirm that the correlation of Rho-GDIα expression and disease-free survival is dependent on the nodal status.

The Rho-GDIα protein is an independent risk factor in invasive ductal breast cancer. Analysis using the Cox hazard regression model for the 90 patient cohort were performed to determine the hazard ratio for the Rho-GDIα protein unadjusted and adjusted for known risk factors (grading, tumor size, nodal and ER status). Between these factors and Rho-GDIα protein expression no significant correlation could be observed as determined by cross table analysis (Table II). Unadjusted, there was a significant 3-fold reduction in the risk for relapse in the group of patients expressing high Rho-GDIα protein levels compared to the group of low expresser (Table II). The hazard ratio only marginally changed and was still statistically significant when adjustment for the other risk factors were done. Note that, in the multivariate analysis, of the risk factors analyzed only Rho-GDIα protein expression and the ER status had a significant impact on the hazard ratio. This suggests that Rho-GDIα protein is an independent risk factor for the clinical outcome of breast cancer patients who receive adjuvant treatment.

Discussion

The analysis of Rho-GDIα RNA expression in invasive ductal breast cancer revealed that untreated patients, in particular with axillary node involvement, tend to progress faster when Rho-GDIα expression was high. In contrast, in patients that received adjuvant treatment, higher Rho-GDIα expression tend to correlate with a better clinical outcome. The latter correlation was statistically significant in a subgroup of CMF-only treated patients. Similar data were obtained

when Rho-GDIα protein expression was determined immunohistochemically in specimens derived from a different group of breast cancer patients. Here again, high Rho-GDIα expression indicated more favorable clinical outcome in the CMF-only treated subgroup, whereas it was not linked to prognosis in the anti-estrogen-only treated subgroup. Interestingly, high Rho-GDIα protein expression was also significantly associated with a better prognosis when all treated patients were included in the analysis (Fig. 4A). This was not found with the Rho-GDIα RNA measurement (Fig. 2A). The reason for this discrepancy may lie in the fact that, in the immunohistochemical analysis, Rho-GDIα was only determined in the tumor cells, whereas in the RT-PCR analysis, Rho-GDIα expression was measured in tumor extracts that included both tumor and stromal cells. Since Rho-GDIα expression in stromal cells and/or the fraction of stromal cells in tumors may vary, stromal Rho-GDIα expression may have an impact on the outcome of the analysis.

By using Cox-regression analysis we also show that, in treated patients, the Rho-GDIα protein is an independent risk factor of relapse, reducing the hazard ratio by 3-fold. The result that high levels of Rho-GDIα indicates a better outcome for CMF-treated patients was unexpected, since a number of cell culture experiments showed that Rho-GDIα protects (rather than sensitizes) breast cancer cells against drug-induced apoptosis (12) and that overexpression of this protein is linked to drug-resistance of ovarian cell lines (11). However, it is not unusual that clinical and cell culture data lead to opposite conclusions on the function of a given protein; e.g., PAI-1 (plasminogen activator inhibitor-1) as an inhibitor of uPA (urokinase plasminogen activator), a key invasion-promoting protease, was first suspected to be a tumor suppressor protein (16). This could not be confirmed by clinical trials, where high PAI-1 expression levels were found to be associated with an unfavorable prognosis in a number of human cancers, including breast cancer (11). PAI-1 is now believed to promote tumor progression by stimulating angiogenesis. Multiple functions have also be attributed to Rho-GDIα. Rho-GDIα has been reported to contribute to ER-activated transcription by directly binding to ERα and its co-activator GRIP1 and by antagonizing ER-repressing Rho-GTPases (17,18). Since ER-negative MCF-7 breast cancer cells are less susceptible to chemotherapeutics than ER-positive MCF-7 cells (19), it is possible that Rho-GDIα sensitizes breast cancer cells to chemotherapeutics by raising ER activity. Alternatively, Rho-GDIα may block the anti-apoptotic functions of certain Rho-GTPases, such as Rac2 and Rho (20,21). Also Rac1 has been reported to act as a survival factor (22) either by activating NF-κB (23) or by inhibiting the pro-apoptotic factor BAD (24). However, it cannot be excluded that Rho-GDIα is not causally linked to CMF sensitivity in breast cancer patients and instead is a surrogate marker for drug resistance.

Little is known about the regulation of Rho-GDIα expression levels. One report shows that G-CSF (granulocyte colony-stimulating factor) increases Rho-GDIα expression in neutrophils (24,25). Interestingly, plasma levels of G-CSF are often elevated in breast cancer (24). Hence, it may be that this cytokine may have an impact on the Rho-GDIα levels in breast cancers.

Since Rho-GDIα and Rho-GDIß have similar abilities to inhibit RhoGTPase and since Rho-GDIß is also expressed in breast cancer specimens (9), we wondered whether Rho-GDIß would have a significant impact on the correlation between Rho-GDIα and prognosis. Our data show that, under certain conditions, this seems to be the case. At low RNA expression of Rho-GDIß, the link between high Rho-GDIα expression and unfavorable prognosis of untreated breast cancer patients is more pronounced (Fig. 1B). In contrast, Rho-GDIß did not influence the correlation of Rho-GDIα and outcome in the group of treated patients. These data are consistent with the notion that, besides sharing functions, the two RhoGDIs may also have individual, not replaceable activities which may be different in the treated vs. untreated setting.

In summary, an important conclusion of our data is that immunohistochemical analysis of the Rho-GDIα protein may be a suitable tool to easily assess the likelihood of a successful response to adjuvant chemotherapy in breast cancer.

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