Prognostic Value of Rho GTPases and Rho Guanine Nucleotide Dissociation Inhibitors in Human Breast Cancers

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ABSTRACT

Purpose: **Rho family members are small GTPases that are known to regulate malignant transformation and motility of cancer cells. The activities of Rhos are regulated by molecules such as guanine nucleotide dissociation inhibitors (GDIs). This study determined the levels of expression and the distribution of Rho-A, -B, -C, and -G, and Rho-6, -7, and -8, as well as Rho-GDI-β, and Rho-GDI-γ, in breast cancer and assessed their prognostic value.**

Experimental Design: **The distribution and location of Rhos and RhoGDIs were assessed using immunohistochemical staining of frozen sections. The levels of transcripts of these molecules were determined using a real-time quantitative PCR. Levels of expression were analyzed against nodal involvement and distant metastasis, grade, and survival over a 6-year follow-up period.**

Results: **The levels of Rho-C, Rho-6, and Rho-G were significantly higher in breast cancer tissues ($n = 120$) than in background normal tissues ($n = 32$). However, the level of Rho-A and –B and rho-7 and -8 was found to be similar in tumor and normal tissues. Immunohistochemical staining revealed the high level of staining of Rho-C protein in tumor cells. The levels of Rho-GDI-γ transcripts were found to be significantly lower in tumor tissues than in normal tissues ($P < 0.05$ and $P < 0.001$, respectively). Node-positive tumors have significantly higher levels of Rho-C and Rho-G, and lower levels of Rho-GDI and Rho-GDI-γ transcripts, than do node-negative tumors. Significantly higher levels of Rho-C and Rho-G were seen in patients who died of breast cancer than in those who remained disease free. Patients with recurrent disease, with metastasis or who died of breast cancer, also exhibited higher levels of Rho-6 but lower levels of Rho-GDI-γ. Higher-grade tumors were also associated with low levels of Rho-GDI and Rho-GDI-γ.**

Conclusions: **Raised levels of Rho-C, Rho-G and Rho-6 and reduced expression of Rho-GDI and -GDI-γ in breast tumor tissues are correlated with the nodal involvement and metastasis. This suggests that the expression of Rhos and Rho-GDIs in breast cancer is unbalanced and that this disturbance has clinical significance in breast cancer.**

INTRODUCTION

Breast cancer affects 1 in 10 women in their lifetime in the United States and the United Kingdom. The single most important factor that affects the prognosis of these patients is the presence and development of metastasis. The development of metastasis is known to be associated with many factors such as angiogenesis, lymphangiogenesis, and the invasiveness and motile nature of breast cancer cells. The latter is known to be one of the most important factors that affect the invasive nature of breast cancer cells. One of the factors that determines the metastatic nature of cancer cells is the motility of cancer cells, which is controlled by exogenous factors as well as by intracellular events. Factors known to increase the motility of cancer cells include hepatocyte growth factor (scatter factor), motility-stimulating factor, autotaxin, and a few other cytokines, collectively known as motogens. Although these factors each have their specific receptors and receptor signaling pathways, it appears that a group of small intracellular molecules, *i.e.,* the rho/rac family, are among the common regulators of cell motility induced by these motogens.

The Rho family belongs to the *ras* oncogene family and is composed of a number of small, highly homologous protein members (for a recent review, see Refs. 1 and 2). The family is known to have A, B, and C isoforms that are highly homologous in their sequence, and other newly identified members including Rho-G; Rho-6, -7, and -8; Rac3; and CDC42. Rho proteins are known to exist in two states: the active-GTP-bound Rho and the inactive-GDP-bound Rho (2–5). The regulation of the activation and inactivation of Rhos is governed by a panel of inhibitors and activators (1, 2). The activators such as the guanine exchange factor allow conversion of GDP-bound Rhos to GTP-bound Rhos, thus becoming the active-form Rhos (3–5). The inhibitors to the Rhos include guanine nucleotide dissociation inhibitors (GDIs) that prevent GTP from being dissociated from the Rho, thus preventing Rhos from being recycled. Rho family members are known to be involved in a number of biological processes, including cell transformation, cell motility, and cell adhesion, primarily by regulating the cytoskeleton system (6–8). The rho/rac family is particularly involved in cell migration and membrane ruffling induced by motogens, such as epidermal growth factor and hepatocyte growth factor (6, 9–12).

In cancer cells, Rho proteins have been demonstrated to

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play key roles in mediating cellular migration, cell adhesion, and cell growth (13, 14, 15, 16). The involvement of Rho molecules in human cancer has been explored in recent years. In pancreatic cancer, Rho-C was found to be overexpressed at the protein level and have some links to clinical outcome (17, 18). Transfection of Rho-C in mammary epithelial cells induces the invasive growth of breast cancer cells (18). In liver cancer, it has also been shown that rho-B is aberrantly expressed in cancer tissues (19). In testicular germ cell tumors, Rho-A and rho-kinase were found to be overexpressed (20). In pancreatic cancer, Rho-C was overexpressed and linked to metastasis and prognosis (21).

In breast cancer, there are some limited reports showing that there are also aberrant levels of Rho-C in tumor cells and tissues. For example, Rho-C is overexpressed in inflammatory breast cancer tissues. In a recent study, when comparing the genes differentially expressed in metastatic breast tumors using microarray, we found Rho-C to be one of the molecules that is profoundly overexpressed in metastatic tumors compared with the primary tumors (22–23).

Most of the knowledge of the Rho family in clinical cancer, limited by the small number of reports, involves Rho-A, -B, and -C. It is not known whether other Rho members also play a role in cancer. There have been no reports in the literature studying the role and the changes of rho regulators, such as rho-GDIs in human cancer. Furthermore, although it has been suggested that Rhos may also be involved in metastasis (5, 24), currently, there is little clinical study to support this. The present study examined the level of expression of a panel of rho family members, including rho-A, -B, -C, -G, -6, -7 and -8, and in particular rho-GDI-α, -β and -γ, in a cohort of patients with breast cancer. Here, we report the aberrant expression of rho proteins and, for the first time, the aberrant expression of Rho-GDI-α and -γ, in breast cancer and its association with clinical outcome.

Table 1 Clinical information on patients in the study

	n
Node status	
Node negative	65
Node positive	55
Grade	
1	23
2	41
3	56
Histology	
Ductal	88
Lobular	14
Others	
Medullary	2
Tubular	2
Mucinous	4
TNM[a] staging	
1	69
2	40
3	7
4	4
Clinical outcome	
Disease free	87
With metastasis	6
With local recurrence	5
Died of breast cancer	16
Died of unrelated disease	6

[a] TNM, tumor-nod-metastasis.

Table 2 Primer sequences

	Sense primer (5′-3′)	Antisense primer (5′-3′)
Rho A	ctgctctgcaagctagacg	caagacaaggcaaccagat
Rho B	gctacggctcccagaac	atagcaccttgcagcagtt
Rho C	gagaagtggaccccagag	cttcatcttggccagctc
Rho G	ctgctcatctgctacacaac	cgctgtaattgtcgaacac
Rho 6	gatgtaagctcgttctggtc	atagcaatccttcgctaaca
Rho 7	ggcttatccctgttacacat	actgtagccacatggaagac
Rho 8	tagagctctccaatcacagg	acgctattttccgactgtaa
Rho GDI[a]	cagattgcagcggagaac	ctctcgtcgtccttgtcc
Rho GDI-β	caattataagcctccaccac	agcagcgttttcttgtactt
Rho GDI-γ	agtcctcctggctgacaa	cacagcctcatccaacac

[a] GDI, guanine nucleotide dissociation inhibitor.

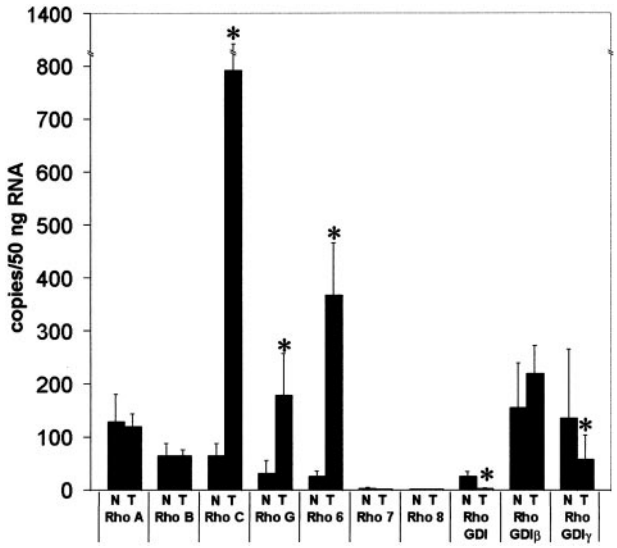

Fig. 1 Levels of expression of Rhos in tumor (*T*) and normal (*N*) mammary tissues. Shown are number (mean ± SD) of copies of respective transcripts from 50 ng total RNA. *, $P < 0.05$ *versus* respective normal tissues. *GDI*, guanine nucleotide dissociation inhibitor.

MATERIALS AND METHODS

Materials. RNA extraction kit and reverse transcription kit were obtained from AbGene Ltd. (Surrey, United Kingdom). PCR primers were designed using Beacon Designer (Palo Alto, CA) and synthesized by Invitrogen Ltd. (Paisley, United Kingdom). Molecular-biology grade agarose and DNA ladder were from Invitrogen. Master mix for routine PCR and quantitative PCR was from AbGene. Rabbit antihuman Rho-A, Rho-B, Rho-C, Rho-G, and Rho-7 and Rho-8 polyclonal antibodies were purchased from Santa Cruz Biotechnology Ltd. (Santa Cruz, CA). Polyclonal antibodies to human rho-GDI, Rho-GDI-β, and Rho GDI-γ were from Santa Cruz Biotechnology Ltd. Peroxidase-conjugated antirabbit and antigoat antibodies were from Sigma and a biotin universal staining kit was from Dako Ltd.

Samples Collection. Breast cancer cell lines MCF-7 and MDA MB 231 were purchased from the European Collection of

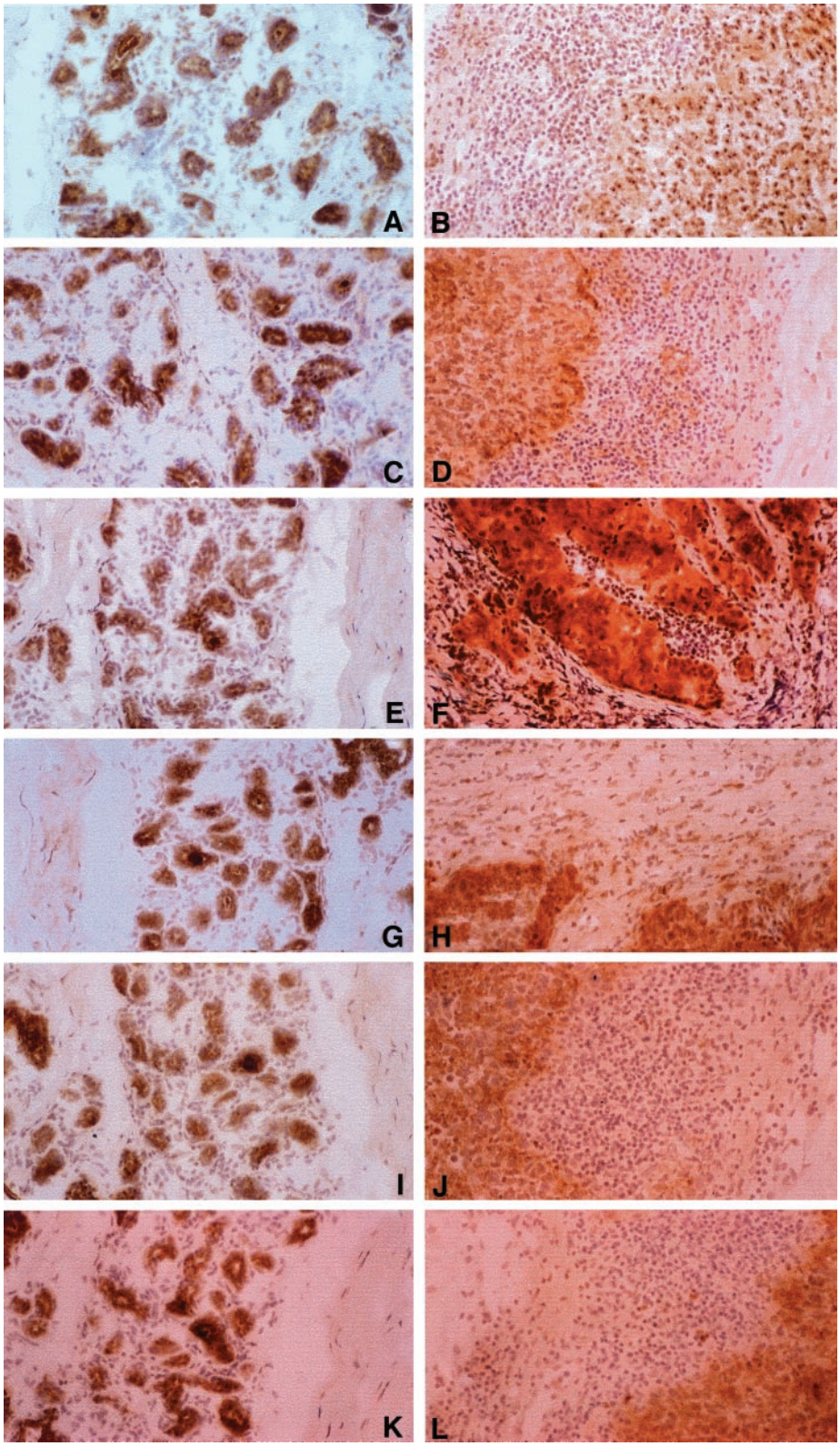

Fig. 2 Immunohistochemical staining of Rhos in background normal (*left panels*) and tumor (*right panels*) tissues, using frozen-sectioned slides. *A* and *B*, Rho-A; *C* and *D*, Rho-B; *E* and *F*, Rho-C; *G* and *H*, Rho-G; *I* and *J*, Rho-7; *K* and *L*, Rho-8.

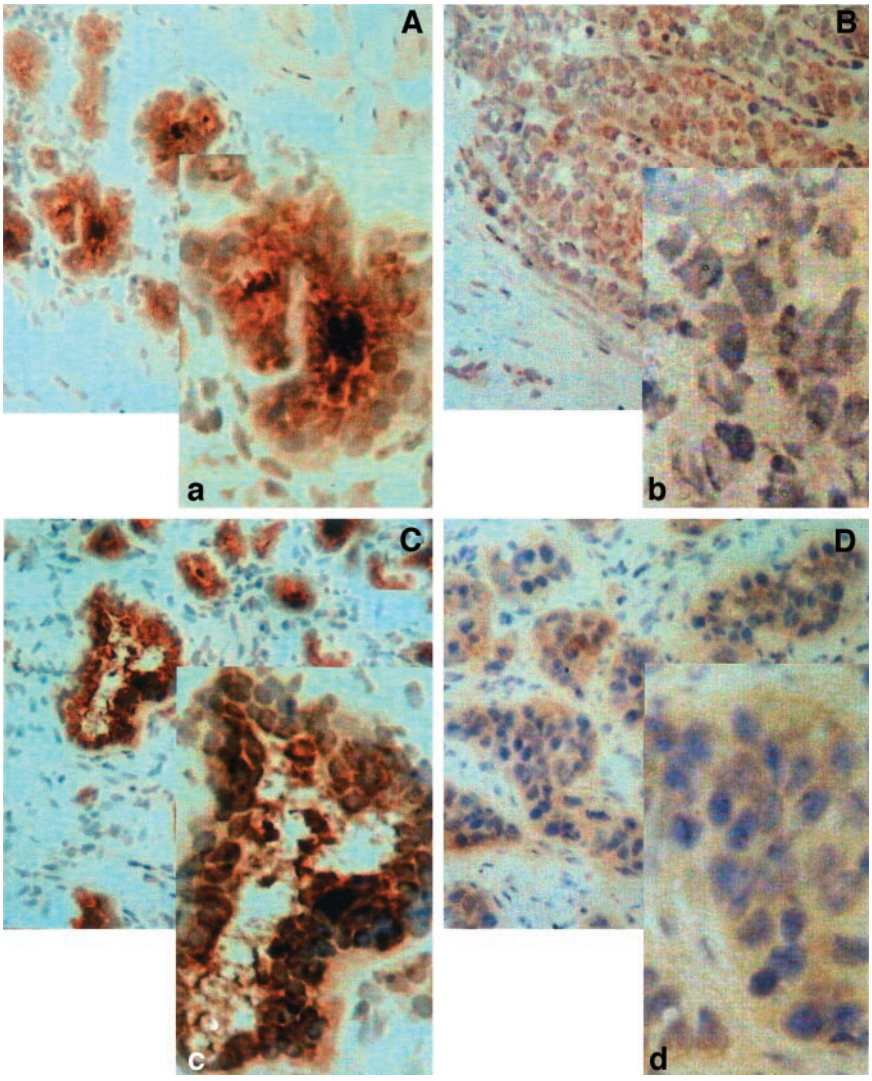

Fig. 3 Immunohistochemical staining of Rho-GDI in tissues. *Left panels,* normal background tissues. *Right panels,* tumor tissues (*A–D,* ×100; *a–d (inserts),* ×250). *A/a* and *B/b,* Rho-GDI; *C/c* and *D/d,* Rho-GDI-γ.

Animal Cell Cultures (ECACC, Salisbury, England). Human umbilical vein endothelial cells (HUVECs) were purchased from TCS Biologicals (Oxford, England). Breast cancer tissues ($n = 120$) and normal background tissues ($n = 32$) were collected immediately after surgery and stored at −80°C until use. Patients were routinely followed clinically after surgery. The median follow-up period was 72 months. The presence of tumor cells in the collected tissues was verified by a consultant pathologist (A. D.-J.), who examined H&E-stained frozen sections.

Tissue from patients with breast cancer who had undergone mastectomy were collected immediately after surgery and stored at −80°C. Details of histology were obtained from pathology reports (Table 1). Patients were routinely followed up on a regular basis and details were stored in a database.

Tissue Processing, RNA Extraction, and cDNA Synthesis. Frozen sections of tissues were cut at a thickness of 5–10 μm and were kept for immunohistochemistry and routine histology. An additional 15–20 sections were mixed and homogenized using a hand-held homogenizer, in ice-cold RNA extraction solution. The concentration of RNA was determined using a UV spectrophotometer. Reverse transcription was carried out using a reverse transcription kit with an anchored oligo(dT) primer supplied by AbGene, using 1 μg of total RNA in a 96-well plate. The quality of cDNA was verified using β-actin primers.

Quantitative Analysis of rho Family Members. The level of rho transcripts from the above-prepared cDNA was determined using a real-time quantitative PCR, based on the Amplifluor technology, modified from a method reported previously (25). Briefly, pairs of PCR primers were similarly designed using the Beacon Designer software (version 2, C; sequence given in Table 2), but to one of the primers, an additional sequence, known as the Z sequence (5′-actgaacctgaccgtaca-3′) which is complementary to the universal Z probe (Intergen Inc., Oxford, United Kingdom), was added. A Taqman detection kit for β-actin was purchased from Perkin-Elmer. The reaction was carried out using the following: Hot-start Q-master

Table 3 Levels of Rho family members in tumor with and without nodal involvement (mean ± SD)

	Node negative	Node positive	*P*
Rho A	76.9 ± 16.1	180.3 ± 52.9	n.s.[a]
Rho B	65.2 ± 17.1	63.2 ± 15.8	n.s.
Rho C	110.6 ± 24.8	1697.0 ± 1434	<0.05
Rho G	77.3 ± 27.6	449 ± 326	<0.01
Rho 6	405 ± 125	370 ± 168	n.s.
Rho 7	1.16 ± 0.57	1.06 ± 0.27	n.s.
Rho 8	0.436 ± 0.219	1.07 ± 0.49	n.s.
Rho GDI[b]	2.94 ± 1.41	1.99 ± 0.48	0.05
Rho GDI-β	126.2 ± 27.3	324 ± 117	n.s.
Rho GDI-γ	98.1 ± 86.6	10.8 ± 4.8	<0.05

[a] n.s., not significant.
[b] GDI, guanine nucleotide dissociation inhibitor.

Table 4 Levels of Rho family members in relation to tumor grade (mean ± SD)

	Grade 1	Grade 2	Grade 3
Rho A	89.4 ± 26.3	118.4 ± 38.3	136.7 ± 44.9
Rho B	88.8 ± 33.2	45 ± 12.1	66.7 ± 19
Rho C	253.3 ± 67.5	2080 ± 1854	111.6 ± 28.7
Rho G	77.2 ± 35.9	312 ± 218[a]	117.9 ± 34.2
Rho 6	167.3 ± 68.8	455 ± 181[a]	343 ± 156[a]
Rho 7	0.66 ± 0.43	1.85 ± 0.91	0.9 ± 0.23
Rho 8	0.13 ± 0.1	0.34 ± 0.17	1.08 ± 0.41
Rho GDI[b]	5.8 ± 4.1	1.8 ± 0.39[a]	1.7 ± 0.43[a]
Rho GDI-β	385 ± 203	121.1 ± 34.1	210.9 ± 74
Rho GDI-γ	241 ± 237	6.8 ± 2.9[a]	19.5 ± 6.5[a]

[a] *Versus* grade 1, $P < 0.05$.
[b] GDI, guanine nucleotide dissociation inhibitor.

mix (Abgene), 10 pmol of specific forward primer, 1 pmol reverse primer which has the Z sequence, 10 pmol of FAM-tagged probe (Intergen Inc.), and cDNA from ~50 ng of RNA (26, 27). The reaction was carried out using IcyclerIQ (Bio-Rad), which is equipped with an optic unit that allows real-time detection of 96 reactions, under the following conditions: 94°C for 12 min and 50 cycles of 94°C for 15 s, 55°C for 40 s, and 72°C for 20 s (27, 28). The levels of the transcripts were generated from a standard that was simultaneously amplified with the samples.

Immunohistochemical Staining of Rho Family Proteins. Frozen sections of breast tumor and background tissue were cut at a thickness of 6 μm using a cryostat. The sections were mounted on SuperFrostPlus microscope slides and were air-dried and then fixed in a mixture of 50% acetone and 50% methanol. The sections were then placed in "Optimax" wash buffer for 5–10 min to rehydrate. Sections were incubated for 20 min in a 0.6% BSA blocking solution and were probed with the primary antibody. After extensive washings, sections were incubated for 30 min in the secondary biotinylated antibody (Multilink Swine anti-goat/-mouse/-rabbit immunoglobulin, Dako Inc.). After washings, Avidin Biotin Complex (Vector Laboratories) was then applied to the sections followed by extensive washings. Diaminobenzidine chromogen (Vector Labs) was then added to the sections, which were then incubated in the dark for 5 min. Sections were then counterstained in Gill's hematoxylin and were dehydrated in ascending grades of methanol before clearing in xylene and mounting under a coverslip.

Statistical Analysis. Statistical analysis was carried out using the Mann-Whitney *U* test and the Kruskal-Wallis test.

RESULTS

Aberrant Expression of Rho-C, Rho-6, and Rho-G in Human Breast Cancer. Varying levels of rho expression in mammary tissues were detected. Whereas Rho-7 and Rho-8 were expressed at relatively low levels, other Rhos existed at higher levels (Fig. 1). Levels of Rho-C, Rho-G, and Rho-6 were significantly raised in tumor tissues compared with normal tissues (Fig. 1). Rho-A, Rho-B, Rho-7, and Rho-8 did not show significant differences between normal and tumor tissues.

Expression of rho-GDI and Rho-GDI-γ Was Reduced in Breast Cancer. Fig. 1 also shows the level of expression of three rho-GDIs in breast tissues. Breast tumor tissues displayed significantly lower levels of Rho-GDI and Rho-GDI-γ than did background normal tissues ($P < 0.05$). The level of Rho-GDI-β remained similar between normal and tumor tissues.

Rho and Rho-GDI Staining in Breast Cancer. Rhos were seen primarily in epithelial cells of the normal mammary tissues (Fig. 2). Stromal tissues stained very weakly with rho. In breast cancer tissues, breast cancer cells stained weakly for Rho-A and Rho-B. In contrast, Rho-C (Fig. 2*F*) and Rho-G (Fig. 2*G*) stained strongly in breast cancer cells, in comparison with normal tissues.

Rho-GDI and Rho-GDI-γ were both highly stained in normal breast tissues (Fig. 3, *A* and *a*, *C* and *c*, respectively). However, breast tumor tissues stained very weakly for both molecules, which is in clear contrast to the staining of normal breast tissues (Fig. 3, *B* and *b*, *D* and *d*, respectively).

Raised Levels of Rho-C, Rho-G, and Rho GDI-γ in Node-Positive Tumors. We have analyzed the levels of Rhos in relation to the nodal status (Table 3). Node-positive tumors had significantly higher levels of Rho-C and Rho-G. These tumors also had significantly lower levels of Rho-GDI and particularly Rho-GDI-γ, compared with node-negative tumors. The level of Rho-6 remained similar between node-positive and node-negative tumors.

Rho and Rho-GDIs and Tumor Staging and Tumor Types. We have examined the expression of Rhos with tumor

Table 5 Levels of Rho family members in tumor according to tumor-node-metastasis (TNM) classification (mean ± SD)

	TNM 1	TNM 2	TNM 3	TNM 4
Rho A	133.9 ± 35.7	106.6 ± 44.9	196.9 ± 76.9	7.1 ± 3.8
Rho B	52.4 ± 11.3	63.7 ± 21.8	223 ± 114[b]	77.5 ± 70.3
Rho C	247 ± 112	2026 ± 1064	205 ± 113	196.6 ± 12.4
Rho G	87.5 ± 21.8	313 ± 234	324 ± 178[b]	288 ± 282
Rho 6	332 ± 111	489 ± 243	415 ± 247	9.4 ± 2.4
Rho 7	1.34 ± 0.57	1.05 ± 0.36	0.58 ± 0.32	0.24 ± 0.09
Rho 8	0.76 ± 0.31	0.43 ± 0.20	0.19 ± 0.17	3.64 ± 3.3
Rho GDI	1.94 ± 0.4	3.9 ± 2.4	2.27 ± 0.79	2.86 ± 2.2
Rho GDI-β	205.3 ± 72.8	209.7 ± 99.1	205 ± 151	747 ± 251
Rho GDI-γ	6.4 ± 2.3	154 ± 140	30.1 ± 24.7	23.0 ± 22

[a] TNM, tumor-node-metastasis; GDI, guanine nucleotide dissociation inhibitor.
[b] $P < 0.05$ *versus* TNM 1.

Table 6 Levels of Rhos in tumor related to tumor type (mean ± SD)

	Ductal	Lobular	Mucinous	Medullary	Tubular	Others
Rho A	132.1 ± 30.8	80.6 ± 47.5[a]	84.2 ± 62.8	2.27 ± 1.1[a]	116 ± 115	71.7 ± 39.6
Rho B	73.8 ± 13.9	26.3 ± 18.9[a]	29.5 ± 22.3	3.1 ± 0.3	8.6 ± 6.9[a]	17.7 ± 11.5[a]
Rho C	118.5 ± 21.9	616 ± 558	334 ± 316	26.4 ± 25.2[b]	247 ± 209	235 ± 159
Rho G	1127 ± 909	32.7 ± 17.2[a]	20.4 ± 11.7	0.91 ± 0.4[a,b]	45.1 ± 36.2	21.7 ± 10.6[a]
Rho 6	417 ± 124	161 ± 81	159 ± 157	4.4 ± 4.07[a,b]	122 ± 120	111.2 ± 79.8[a]
Rho 7	0.9 ± 0.18	3.2 ± 3.0	3.52412.2	0.07 ± 0.072[a]	0.74 ± 0.64	1.95 ± 1.2
Rho 8	0.64 ± 0.21	0.22 ± 0.19	4.43 ± 4.4	0.66 ± 0.65	0.03 ± 0.01[a]	2.1 ± 1.8
Rho GDI[c]	2.9 ± 1.01	0.93 ± 0.38[a]	0.38 ± 0.19	3.3 ± 3.2	1.26 ± 0.68	1.3 ± 0.78
Rho GDI-β	243 ± 67.9	60.5 ± 28.7[a]	35.5 ± 26.8	3.8 ± 3.7[a,b]	407 ± 313[b]	120.3 ± 87.1
Rho GDI-γ	11.3 ± 3.3	9.5 ± 7.4	9.9 ± 9	0.029 ±	0.04 ± 0.003[a]	4.99 ± 4.6

[a] *Versus* ductal.
[b] *Versus* lobular.
[c] GDI, guanine nucleotide dissociation inhibitor.

grade (Table 4), tumor-node-metastasis status (Table 5), and tumor types (Table 6). Significantly higher levels of Rho-6 were seen in grade 2 and grade 3 tumors compared with grade 1 tumors. Conversely, grade 2 and grade 3 tumors exhibited significantly lower levels of Rho-GDI and Rho-GDI-γ**.** Rho-C and Rho-G were otherwise seen at high level in grade 2 tumors. Lobular tumors had lower levels of Rho-A, Rho-B, and Rho-G. They also showed lower levels of Rho-GDI and Rho-GDI-β (Table 5). Rho-C and Rho-G were expressed at a significantly higher level in tumor-node-metastasis 3 tumors. The difference between other stages and Rho molecules were not significant.

Rho and Rho-GDI Correlated with Prognosis. Patients with local recurrence and patients who died of breast cancer had significantly higher levels of Rho-C and Rho-6, markedly rho-G (Fig. 4). Interestingly, significantly lower levels of Rho-GDI-α and Rho-GDI-γ (Fig. 5) were seen. The strikingly high level of Rho-C in patients who died of breast cancer was seemingly biased by the fact that two of the patients demonstrated ex-

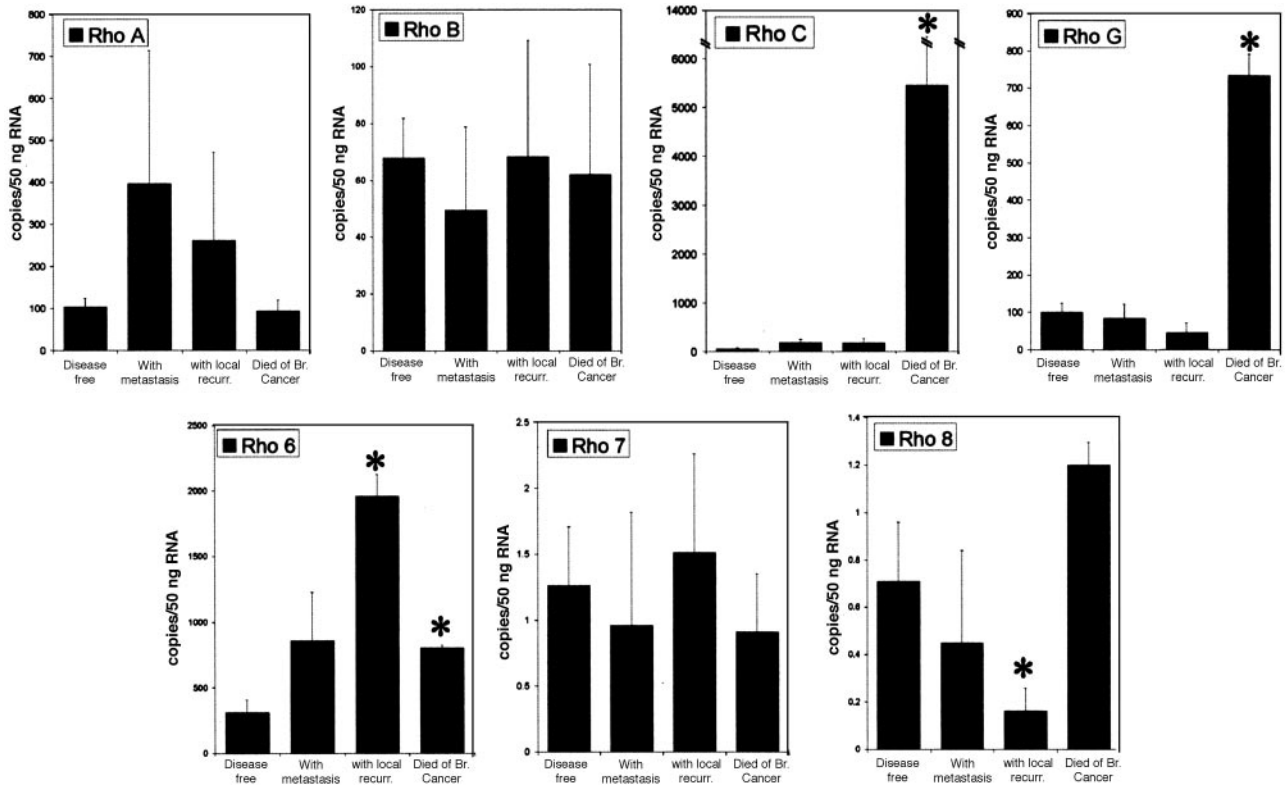

Fig. 4 Rhos and clinical outcomes. Patients were divided into four groups: disease free; with metastasis; with local recurrence (*with local recurr.*); or died of breast cancer (*Died of Br. Cancer*; excluding patients who died of other unrelated diseases). Shown are mean ± SD. ∗, $P < 0.05$ *versus* disease free.

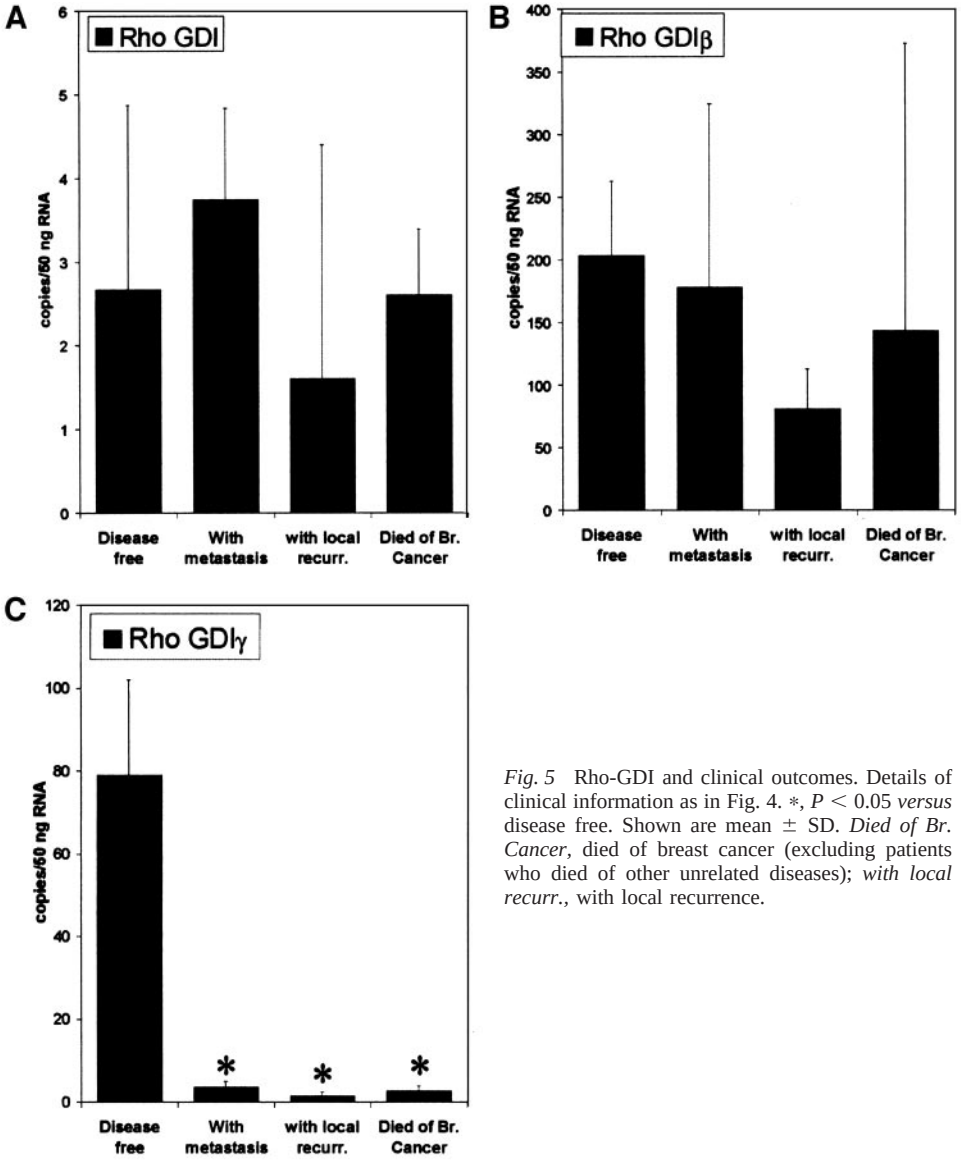

Fig. 5 Rho-GDI and clinical outcomes. Details of clinical information as in Fig. 4. *, $P < 0.05$ *versus* disease free. Shown are mean ± SD. *Died of Br. Cancer,* died of breast cancer (excluding patients who died of other unrelated diseases); *with local recurr.,* with local recurrence.

tremely high levels. However, even if these two patients were considered separately, the remaining patients who died of breast cancer continued to display a significantly higher level of Rho-C (199 ± 82 copies/50 ng RNA, *versus* 58.8 ± 23.2 in disease-free group, $P < 0.05$).

DISCUSSION

The present study reported aberrant expression of rho family members in human breast cancer and, for the first time, the aberrant expression of Rho-GDI in breast cancer. Whereas the levels of Rho-C, Rho-G, and Rho-6 were significantly increased in breast tumor tissues, the levels of Rho-GDI-α and Rho-GDI-γ, however, exhibited a significantly lower level in tumor tissues than in normal tissues. In addition, levels of selective Rho and Rho-GDI are associated with clinical outcome of the patients.

The observation that Rho-C was significantly raised in breast cancer tissues is in line with observations made with other tumor types, such as pancreatic cancer and gastric cancer. This study provides further evidence that Rho-6 is also significantly increased in breast cancer, and, in contrast, the levels of Rho-A, Rho-B, Rho-7, and Rho-8 remained similar between normal and tumor tissues. It appears that there is a general aberrant expression of Rho family members in breast tumor tissues.

This study has revealed for the first time that there is an imbalance between the levels of Rhos and Rho activation inhibitors, GDIs, in breast cancer. In our view, this observation is of particular importance. Rho-GDIs are activation regulators for Rho-GTPases and are known inhibitors to the activation of Rho-GTPases, by preventing the dissociation of GTP from active Rho-GTP. The excessive level of Rho and reduced level of GDI may suggest that, in breast tumor tissues, the increased

levels of the Rhos, C, 6, and G, may remain to be activated, at a constant level, attributable to the inability to deactivate Rho by the loss of Rho-GDIs. This connection is further supported by a recent study that shows that Rho-GDI-2 mRNA is expressed only in low-invasive tumor cell T24 and not in highly invasive T24T cells (29). In ovarian tumors, however, the same Rho-GDI-2 was up-regulated (30).

One of the most interesting observations in the present study is the correlation between certain rho members and nodal involvement and metastasis/mortality. Rho-C has been shown to be one of the most notable molecules in metastatic cancer cells (23). In addition to the proposed role of Rho GTPases in cancer, Rho GTPases have been shown to regulate transcriptional events in breast cancer cells (31). For example, Rho-GDI has been shown to regulate the transcriptional activation of estrogen receptor, progesterone receptor, and glucocortinoid receptor in mammalian cells and to antagonize the effects of Rho-A (31). Furthermore, Rho-C overexpression has been indicated as playing a role in cancer-induced angiogenesis, by inducing the secretion of angiogenic factors, vascular endothelial growth factor, basic fibroblast growth factor, interleukin 6, and interleukin 8, from breast cancer cells (32). Rho is also involved in other molecules that are involved in cancer progression, such as CD44 (33) and E-cadherin (34), and in hepatocyte growth factor-induced invasion (35). Thus, overexpression of Rho-GTPases may contribute to the aggressive nature of breast cancer cells, as well as to tumor-induced biological processes involved in disease progression, including angiogenesis.

Although Rho-C and Rho-A have been previously linked to the promotion of carcinogenesis (36), in contrast, Rho-B has been implicated as playing a tumor suppressor role (37). In addition, the exact biological roles of Rho-G and Rho-6 are yet to be fully established. Rho-G has been recently implicated in the regulation of apoptosis process (38), via phosphatidylinositol 3′-kinase and Akt pathways. It would be interesting to pursue studies to determine the genetic impact of these Rho-GTPases in cancer cells.

Mutation of Rho GTPases in cancer is rare (38, 39). This may suggest that therapeutic agents targeting Rho-GTPases are less affected by the genetic abnormalities widely seen in cancer. Indeed, it has been shown that inhibitors to Rho kinases have been shown to inhibit the migration and invasion of cancer cells (39). The findings from this study and previous reports, thus, indicates that targeting Rho-GTPases and their regulators may be a viable strategy in regulating the aggressive nature of breast cancer cells.

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