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Molecular Epidemiologic Features of Inflammatory Breast Cancer: A Comparison between Egyptian and U.S. Patients

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Abstract

Background—Inflammatory breast cancer (IBC) is a lethal form of breast cancer with unknown etiology. A higher frequency of IBC and a more aggressive IBC phenotype was reported in Egypt than in the U.S. This difference in disease frequency and presentation might be related to molecular epidemiologic factors.

Methods—We used tumor blocks and demographic, epidemiologic, and clinical data of 48 IBC patients from Egypt and 12 patients from the U.S. We counted tumor emboli in tumors before and after immunohistochemical staining with lymphatic vessel endothelial receptor-1 (LYVE-1), and measured the expression of RhoC GTPase protein in the two groups.

Results—Erythema, edema, and peau d'orange were found in 77% of the Egyptian patients as compared with 29% found in the U.S. patients ($P = 0.02$). The number of tumor emboli was significantly higher in tumors from Egypt (mean ± SD, 14.1 ± 14.0) than in the tumors from the U.S. (5.0 ± 4.0, $P = 0.01$). The number of tumor emboli in LYVE-1 positive vessels was higher in tumors from Egypt (3.5 ± 2.8) than tumors from the U.S. (1.6 ± 0.5, $P = 0.15$). We detected a high level of RhoC in 87% of the tumors from Egypt and 14% of the tumors from the U.S. ($P = 0.0003$).

Conclusion—Patients from Egypt have a more aggressive form of IBC than those in the U.S. Our analysis of IBC patients shows that distinct molecular phenotypes can be found when these

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two study populations are compared. Future studies should explore the epidemiologic and environmental exposures and the genetic factors that might lead to the different clinical and molecular features of IBC in patients from these two countries.

Keywords

inflammatory breast cancer; tumor emboli; RhoC; LYVE-1; molecular epidemiology; international

Introduction

Inflammatory breast cancer (IBC) is rare but it is the most lethal form of locally advanced breast cancer. Compared with other types of breast cancer, IBC affects younger women, progresses rapidly, and tends to be more advanced at the time of diagnosis [1–2]. In the U.S., IBC accounts for approximately 2% of newly diagnosed breast cancers and 7% of all breast cancer-specific mortality, with a 5-year survival of less than 40% [3]. Moreover, the incidence of IBC has increased more rapidly over the past few decades when compared with all other types of breast cancer [3–4].

Despite its dismal prognosis, little is known about the etiology of IBC, and the diagnostic criteria of IBC have not been standardized [5]. Studying the molecular basis of IBC might aid in developing accurate diagnostic criteria and improving treatment strategies [6]. Research in Egypt has suggested the IBC signature occurs in 10% of all breast cancer patients. These IBC patients present with a younger age of onset and a more aggressive clinical course than in IBC seen in the U.S. and other countries [7–8]. These findings suggest there might be molecular differences between patients that explain the more aggressive IBC phenotype found in Egypt.

Apart from the clinical presentation, tumor emboli in the dermal area are the most important pathological indicator that point to the diagnosis IBC [9]. The presence of dermal tumor emboli has been associated with positive lymph node involvement and a poor prognosis for IBC patients in several studies [10–12], but this association has not been investigated quantitatively. Furthermore, a high level of an oncogene protein, RhoC GTPase, was reported in IBC tumors from the U.S. [13], but it is not clear if this is also the case in IBC tumors from other ethnic groups.

We conducted an epidemiologic study on a total of 60 IBC patients from Egypt and the U.S. Our aim was to measure the number of tumor emboli and the levels of RhoC in the IBC tumors from the two countries. To determine whether the observed tumor emboli were located in lymphatic vessels, we performed immunohistochemistry (IHC) with an antibody directed against the human lymphatic vessel endothelial receptor-1 (LYVE-1), a receptor found specifically in endothelial cells lining lymphatic vessels [14–15].

Materials and Methods

Study Population

Forty-one IBC patients from the National Cancer Institute of Cairo University (NCI-Cairo) and 7 patients from the Gharbiah Cancer Registry in Tanta, Egypt (September 2005 to December 2006) were included in the study. The diagnosis of IBC was confirmed by a clinical evaluation and histopathologic studies by 5 of the co-authors (SO, HK, SE, CGK, and SDM). The mean age of the 48 Egyptian patients was 46.9 years old (standard deviation, SD = 11.0), with a range of 28–70 years old. Bilateral breast involvement was found in 3 patients. The majority of IBC tumors from the patients from Egypt were invasive

ductal carcinoma (IDC), which was detected in 41 (89.1%) patients. The other histologic types included lobular carcinoma (2 patients, 4.4%) and mixed IDC and lobular carcinoma (3 patients, 6.5%).

Because IBC is a rare disease in the U.S., we were only able to identify IBC tumors from 4 patients from the University of Michigan Comprehensive Cancer Center (UMCCC) and 3 tumors from patients from the Josephine Ford Cancer Center at the Henry Ford Hospital (1992 and 2006). In addition, we located data on a number of tumor emboli from 5 other patients at the UMCCC, but without the associated tumor tissues or clinical data. The mean age of the U.S. patients was 57.3 years old (SD = 13.9) with a range of 36–75 years old. One U.S. patient was African American and all others in that group were Caucasian. Bilateral breast involvement was found in 1 Caucasian patient. The histology of tumors from the patients from the U.S. included IDC (6 patients, 85.7%) and lobular carcinoma (1 patient, 14.3%).

Data Collection

The institutional review boards at the participating institutions in Egypt and the U.S. approved this study. We examined medical records and pathology reports to obtain information on age, menopausal status, parity (number of live births), disease duration, clinical symptoms, and the histopathologic analysis of IBC in the study population. The duration of the disease was the self-reported time between the onset of IBC-related symptoms and the first visit to a clinician and diagnosis. Exhibition of IBC-related symptoms, including erythema, edema, peau d'orange, palpable mass, and ulceration, was expected to elicit, along with information on pathological factors such as histology, the diameter of the tumor, grade, lymph node involvement and the expression status of estrogen receptor (ER) and progesterone receptor (PR). However, not all desired information was available in the source records. The factors that were not mentioned in records were considered to be missing in those instances and were not considered.

Tissue specimens

Archived tumor samples were retrieved from the recruiting hospitals to determine the histopathology of IBC in the study population. We obtained at least 1 hematoxylin and eosin (H&E) slide from each of the 60 IBC patients to count number of tumor emboli present. The epidermis was not visible in tumor resections from 13 patients from Egypt. However, since these samples were collected prospectively and were known to be from areas close to the skin, they were still included in the analysis. The locations with the highest density of tumor emboli were identified at 10× (objective) magnification, and the average number of tumor emboli was calculated in a mean number of 4 (range 1–5) target areas on each slide.

Immunohistochemistry (IHC)

Polyclonal antibodies from rabbit, directed against human LYVE-1 (Abcam, Inc., Cambridge, Massachusetts, USA), and from chicken, directed against RhoC [16], were used for immunohistochemical analysis of formalin-fixed paraffin-embedded tissues. We were able to examine the expressions of LYVE-1 in 55 patients and RhoC in 54 patients. We used the IHC protocol reported earlier[16] with only minor adjustments. In brief, slides were deparaffinized in xylene, dehydrated in 100-70% ethanol, placed in citrate buffer for 10 minutes and heated. The LYVE-1 antibody was diluted 1:200 and used for staining overnight at 4°C. The RhoC IHC was performed using a 1:3000 dilution for 30 minutes at room temperature. Human post-transplant lymphoproliferative disorder and tumor xenografts from a cell line known to overexpress RhoC (SUM 149) were used as the positive control for LYVE-1 and RhoC, respectively. Appropriate negative controls were used in each analysis.

We defined tumor emboli located in LYVE-1 positive vessels as *lymphatic invasion*, and applied the same method that we used on the HE slides to calculate the average number of tumor emboli in LYVE-1 positive vessels at areas with the strongest signal using the 10× objective magnification. The percent of lymphatic emboli was calculated as the average number of tumor emboli located in LYVE-1 positive vessels divided by the average number of tumor emboli present on the H&E slide for 22 Egyptian and 3 U.S. patients who had positive LYVE-1 expression. Levels of RhoC were scored in the range 1–4 according to the system developed by Kleer et al. in 2005 [17], and the results were expressed as low (score 1 and 2) and high (score 3 and 4) because of the relatively small number of patients in each stratum.

Statistical Analysis

We used descriptive methods to investigate differences between IBC patients from Egypt and the U.S. Typical symptoms of IBC include erythema, edema, and peau d'orange [6]. Therefore, patients who had all 3 symptoms were grouped into a single stratum to be compared with patients who had fewer than 3 symptoms. The Fisher's exact probability test and the Mann-Whitney's U-test were used to evaluate differences in epidemiologic, clinical, histopathologic, and molecular characteristics between the Egyptian and the U.S. patients. Because of the relatively small sample size, we combined patients with any positive expression of either ER or PR into one group and compared them with the double negative group. Given the larger size of the Egyptian patient study sample, we performed both parametric (mean, SD, range, and Pearson's correlation coefficient) and non-parametric (median and Spearman rank correlation coefficients) tests to explore potential associations between the duration of symptoms, tumor size, and number of tumor emboli in patients from Egypt [18–19]. In addition, we computed the non-parametric one-way ANOVA to study correlations between categorical factors (RhoC level and the number of the core symptoms presented at diagnosis) and continuous factors (duration of symptoms, tumor size, and the number of tumor emboli). We used the Fisher's exact test to evaluate the correlation between RhoC levels and number of symptoms in IBC patients from Egypt. We did not conduct a similar analysis for the U.S. patient group because of the limited sample size. All tests were two-tailed and computed using the SAS statistical package (SAS v9.1).

Results

As shown in Table 1, the Egyptian patients were younger than the U.S. patients were (P = 0.06, Table 1). Histochemical analysis showed that IBC tumors from Egyptian patients were larger (7.2 ± 3.4 cm) and moderately differentiated (80.4%) when compared with IBC tumors from the U.S. patients (4.7 ± 2.4 cm, P = 0.08 for tumor size; 28.6% for moderate differentiation, P = 0.01, Table 1). We found no significant difference between Egyptian and U.S. patients in menopausal status, parity, combined ER/PR status, or lymph node involvement (Table 1).

The duration of clinical symptoms before diagnosis tended to be longer in patients from Egypt (8.3 ± 10.6 months) than in the patients from the U.S. (2.1 ± 2.3 months, Table 2). At diagnosis, 37 Egyptian patients (77.1%) showed all 3 hallmark symptoms of IBC, but only 2 U.S. patients did (28.6%, P = 0.02, Table 2). The presence of a palpable mass or ulceration at diagnosis was not significantly different between the 2 groups. Eighty-three percent of the Egyptian patients presented with a palpable mass (vs. 71.4% of the US patients) and 11.9% of the Egyptian patients presented with ulceration (vs. 100.0% of the US patients) (Table 2).

Interestingly, the number of tumor emboli was higher in IBC tumors from Egypt (14.1 ± 14.0) compared with that found in IBC tumors from the U.S. (5.0 ± 4.0, P = 0.01, Table 3). Excluding the 13 Egyptian patients for whom no skin was present on the slides did not

change the result ($P = 0.02$, data not shown). Tumor emboli located in LYVE-1 positive lymphatic vessels were only found in 22 (48.9%) and 3 (42.9%) of the IBC tumors from Egypt and the U.S. patients, respectively ($P = 1.00$, Table 3). Failure of LYVE-1 staining was not likely to explain this because all IBC tumors tested showed internal positive staining for LYVE-1. Consistent with our finding of tumor emboli on H&E slides, the average number of tumor emboli located in LYVE-1 positive vessels was higher in IBC tumors from Egypt (3.5 ± 2.8 in the Egyptian group vs. 1.6 ± 0.5 in the U.S. group, $P = 0.15$, Table 3). The average proportion of tumor emboli in LYVE-1 positive vessels observed in each tumor was similar between the 2 groups (42.2% in the Egyptian group vs. 47.3% in the U.S. group, $P = 1.00$, Table 3), which also suggests that the distribution of tumor emboli was similar on H&E slides and LYVE-1 slides. In IBC tumors where the RhoC GTPase level was examined, 87.0% of tumors from Egyptian patients and 14.3% of tumors from the U.S. patients showed high levels of RhoC ($P = 0.0003$, Table 3).

We then performed both parametric and nonparametric tests to further address correlations between variables within the Egyptian group. We found some evidence to suggest that the duration of symptoms was inversely associated with the tumor size (Pearson's correlation coefficient = −0.59, $P = 0.02$), but not with the number of tumor emboli (Pearson's correlation coefficient = −0.01, $P = 0.97$), the RhoC expression level ($F = 0.70$, $P = 0.51$), or the number of the core symptoms in patients from Egypt ($F = 0.09$, $P = 0.91$, data not shown). High levels of RhoC was more likely to be found in smaller IBC tumors ($F = 6.69$, $P < 0.01$) and in Egyptian patients with all 3 hallmark symptoms of IBC ($P < 0.01$). We did not find an association between the number of tumor emboli and the tumor size, the RhoC level, or the number of symptoms in the Egyptian group (data not shown).

Discussion

The findings of this study suggest that IBC as found in Egypt patients is a more aggressive form than IBC as found in patients from the U.S. Patients from Egypt were younger that the patients from the U.S. and had more advanced clinical signs of IBC at diagnosis. In addition, IBC tumors from the Egyptian patients had a higher number of tumor emboli and an elevated level of RhoC, which might suggest that there is a greater potential for metastasis in IBC patients from Egypt than the U.S.

Population-based epidemiological studies show that the mean age of IBC diagnosis in the U.S. is between 57 and 59 years old [2–4]. This is similar to our observation in the U.S. group where the mean age of the onset of IBC was 57.3 years. Although the age of IBC onset has not been well documented in Egypt, previous research on 73 IBC patients from the NCI-Cairo study revealed a median age at diagnosis of 42 years old [8]. Thus, our findings were consistent with previous studies in both countries and indicate that Egyptian patients are younger at the onset of IBC than their counterparts in the U.S.

We also observed a moderately longer duration of symptoms before diagnosis among the Egyptian patients in this study compared with patients from the U.S. The longer duration of symptoms before the initial clinical consultation among the Egyptian patients is less likely to reflect the slower progress of IBC development, but rather a delay in seeking medical care [8]. This phenomenon is common in women from Arab countries. About 28% of breast cancer patients in Egypt report seeking medical care no earlier than 6 months after their observation of a breast mass or lymph node enlargement [20–21]. This tendency to delay medical care may bias the estimate of disease duration for IBC patients in Egypt and may influence the correlation between clinical and molecular phenotypes. However, we did not find evidence suggesting that the longer duration of symptoms in this study was associated with the concurrence of the 3 clinical symptoms characteristic of IBC (erythema, edema, and

peau d'orange), the higher number of tumor emboli, or the elevated RhoC expression seen in IBC patients from Egypt.

Clinically, IBC is characterized by diffuse erythema, edema, and peau d'orange. This inflammation-like presentation of IBC has been causally related to dermal lymphatic invasion by tumor emboli, as opposed to infiltration of inflammatory cells [22]. Because skin biopsies may not show dermal lymphatic emboli, positive observation of tumor emboli has been found in about 25–60% of IBC tumors [10, 23]. The presence of tumor emboli has been associated with positive lymph node involvement, and the co-occurrence of tumor emboli and inflammatory clinical symptoms and has been linked to an undesired outcome in IBC patients [10–12]. Taken together, these findings indicate that an increased number of tumor emboli in IBC tumors might be correlated with a more extensive clinical presentation and a greater potential for tumor metastasis culminating in a lower survival rate. In the current study, we observed that Egyptian patients presented with more symptoms and more tumor emboli than patients from the U.S. did, but we did not find a positive correlation between the number of tumor emboli and the number of symptoms in IBC from Egypt.

Given the moderate to high prevalence of tumor emboli in IBC tumors, the lymphatic system may be an important pathway for metastasis in IBC patients. LYVE-1 is a novel marker commonly used to study lymphangiogenesis [15]. Van der Auwera et al. [24] detected significantly higher levels of LYVE-1 mRNA and protein expression in IBC tumors than in stage-matched non-IBC tumors, indicating that increased expression of LYVE-1 may be associated specifically with IBC. However, another study by the same group showed only a weak expression of LYVE-1 in intratumoral lymph vessels that were detected by IHC [25]. Interestingly, we found that only 49% of the tumors from Egypt patients and 43% of tumors from the U.S. patients had tumor emboli located in LYVE-1 positive vessels. Among these patients, the number of tumor emboli in LYVE-1 positive lymphatics was higher in the tumors from Egypt than in the tumors from the U.S., while the percentage of tumor emboli in LYVE-1 positive lymphatics was similar in the two groups. False negative staining of LYVE-1 was not likely to explain our observations because strong positive staining of empty lymphatic vessels in peri-tumoral and/or intratumoral areas was found in all IBC patients with tumor emboli located in LYVE1 negative vessels. Tumor emboli in LYVE-1 negative epithelial vessels might be located in LYVE-1 down-regulated lymphatics [26], or in vessels other than lymphatics. In future research, additional histopathologic markers for lymph vessels should be used when studying lymphatic vasculature in IBC tumors.

The oncogenic function of the RhoC protein has been elucidated in IBC and RhoC is consistently associated with different types of advanced cancer [17, 27–32]. Comparative microarray analysis of overexpressed-RhoC and wild-type RhoC cell lines showed that RhoC overexpression can affect the expression of more than one hundred genes that are known to be involved in various biological functions such as enhancing cell cycle progression, angiogenesis, lymphangiogenesis, and cell adhesion and invasion [33]. These findings suggest that the overexpression of RhoC may be responsible for the dismal outcome in IBC patients. A report by van Golen and his colleagues showed that RhoC was overexpressed in 90% of 29 IBC tumors and 38% of 19 non-IBC tumors ($P = 0.0095$) [13]. In the current study, we found a high level of RhoC overexpression in 1 of 7 (14%) IBC tumors from the U.S., as compared with 40 of 46 (87%) IBC tumors from Egypt, indicating that the RhoC GTPase might be an important contributor to IBC in the patients from Egypt, a matter that is worthy of further investigation.

An unusually high proportion of IBC among all breast cancers, a tendency to early age of onset, and a more aggressive clinical course of IBC in patients from Egypt [7–8] than in

patients from the U.S. and other countries underscores the possibility of distinct IBC risk factors in Egypt. These features of IBC may support our notion that patients in Egypt are exposed to unique environmental and genetic factors [34–35]. In our earlier studies, we found that women with no lactation history had a significantly higher level of dichlorodiphenyldichloroethylene (DDE) than women who breast fed ($P = 0.002$) [34]. Age at first childbirth (younger vs. older) were also associated with higher levels of serum DDE concentrations in premenopausal women [34]. Living in urban areas (odds ratio, OR = 3.1, 95% confidence interval, 95% CI = 1.1–9.3), infertility (OR = 9.8, 95% CI = 1.1–89.7), and oxidative exposures, as shown by the presence of 7,8-dihydro-8-oxo-2'-deoxyguanine (8-oxo-dG) in lymphocyte DNA (OR = 5.8, 95% CI = 1.9–17.5), correlate with a higher risk of breast cancer for women in Egypt [35]. Further investigations should be conducted to explore the potential environmental exposures and lifestyle factors that may correlate with the incidence of IBC in different countries.

This study has several strengths. First, the diagnosis of IBC in all patients was confirmed using the same criteria by both clinical and histopathologic methods. Second, the higher incidence of IBC in the large sample of Egyptian patients in this study provides a unique opportunity to investigate the etiology and molecular and clinical profiles of IBC. This may provide insight into characteristics of the disease and reveal potential therapeutic targets. To our knowledge, this is the first study where tumor emboli in IBC tumors were examined quantitatively, and the first report to demonstrate the difference in the number of tumor emboli and RhoC expression between two distinct ethnic and racial groups. The characteristics of the IBC patients recruited in this study were consistent with previous those reported in previous research reports from Egypt [7–8] and the U.S. [2–4, 36–37]. This suggests that the results of the current study are not likely to be affected by selection bias. Nevertheless, the study also had a limitation in that the low incidence of IBC limited the number of patients recruited from the U.S. resulting in a relatively small sample size in that group.

Future studies should build upon the results of this pilot study and investigate the correlation between epidemiologic and environmental risk factors, and the clinical, genetic, and molecular profiles associated with IBC. Since universal well-standardized clinical diagnostic criteria of IBC are still lacking [5], more large international studies could help to elucidate the molecular profile associated with IBC and help to improve diagnostic criteria and the ability to predict IBC disease outcomes.

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Table 1

Characteristics of the study population

	Egypt (n = 48)		**U.S. (n = 12)**		
	No.	**(%)**	**No.**	**(%)**	***P*-value**
Age[48, 7]					
Median, Min – Max	43, 28 – 70		59, 36 – 75		0.06[a]
Mean ± SD	46.9 ± 11.0		57.3 ± 13.9		
Menopausal status[48, 4]					
Pre-	30	(62.5)	1	(25.0)	
Post-	18	(37.5)	3	(75.0)	0.29[b]
Parity[42, 3]					
Median, Min – Max	3, 0 – 7		2, 2 – 3		0.76[a]
Mean ± SD	2.6 ± 1.8		2.3 ± 0.6		
Tumor size (cm)[29, 6]					
Median, Min – Max	8.0, 1.0 – 14.0		4.5, 2.2 – 7.5		0.08[a]
Mean ± SD	7.2 ± 3.4		4.7 ± 2.4		
Differentiation[46, 7]					
Moderately	37	(80.4)	2	(28.6)	
Poorly/Undifferentiation	9	(19.6)	5	(71.4)	0.01[b]
ER/PR[31, 7]					
Negative	13	(41.9)	3	(42.9)	
Positive	18	(58.1)	4	(57.1)	1.00[b]
Lymph node involvement[38, 6]					
Negative	2	(5.3)	1	(16.7)	
Positive	36	(94.7)	5	(83.3)	0.36[b]

[a] Mann-Whitney's U-test;

[b] Fisher's exact test

Table 2

Clinical symptoms of IBC patients from Egypt and the U.S.

	Egypt (n = 48)		U.S. (n = 12)		
	No.	**(%)**	**No.**	**(%)**	***P*-value**
Duration of symptoms (months)[27, 5]					
Median, Min – Max	4.0, 0.5 – 48.7		1.0, 0.6 – 6.0		0.12[a]
Mean ± SD	8.3 ± 10.6		2.1 ± 2.3		
Erythema[48, 6]					
Yes	45	(93.8)	5	(83.3)	
No	3	(6.2)	1	(16.7)	0.38[b]
Edema[48, 5]					
Yes	46	(95.8)	5	(100.0)	
No	2	(4.2)	0		1.00[b]
Peau D'orange[42, 6]					
Yes	39	(92.9)	4	(66.7)	
No	3	(7.1)	2	(33.3)	0.11[b]
Clinical symptoms[48, 7] [c]					
With all 3 symptoms	37	(77.1)	2	(28.6)	
With 1 or 2 symptoms	11	(22.9)	5	(71.4)	0.02[b]
Palpable mass[42, 7]					
Yes	35	(83.3)	5	(71.4)	
No	7	(16.7)	2	(28.6)	0.60[b]
Ulceration[42, 1]					
Yes	5	(11.9)	1	(100.0)	
No	37	(88.1)	0		0.14[b]

[a] Mann-Whitney's U-test;

[b] Fisher's exact test;

[c] The first group included cases with all 3 symptoms of erythema, edema, and peau d'orange, and the other group included all other cases with clinical information available.

Table 3

Number of tumor emboli and LYVE-1 and RhoC expression in IBC tumors from Egypt and the U.S.

	Egypt (n = 48)		U.S. (n = 12)		
	No.	**(%)**	**No.**	**(%)**	***P*-value**
Tumor Emboli[46, 12]					
Median, Min – Max	10.4, 1.0 – 62.2		3.9, 2.0 – 16.2		0.01[a]
Mean ± SD	14.1 ± 14.0		5.0 ± 4.0		
LYVE-1[45, 7]					
Positive	22	(48.9)	3	(42.9)	
Negative	23	(51.1)	4	(57.1)	1.00[b]
# of emboli in LYVE-1 vessels[22, 3]					
Median, Min – Max	2.8, 1.0 – 11.0		1.8, 1.0 – 2.0		0.15[a]
Mean ± SD	3.5 ± 2.8		1.6 ± 0.5		
% of emboli in LYVE-1 vessels[22, 3]					
Median, Min – Max	35.2, 2.5 – 100.0		27.3, 23.8 – 90.9		1.00[a]
Mean ± SD	42.2 ± 33.1		47.3 ± 37.8		
RhoC expression level[46, 7]					
Low	6	(13.0)	6	(85.7)	
High	40	(87.0)	1	(14.3)	0.0003[b]

[a] Mann-Whitney's U-test;

[b] Fisher's exact test