Bioinformatics analysis of potential therapeutic targets among ARHGAP genes in breast cancer

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Abstract. GTPase activating proteins (RhoGAPs) serve significant roles in multiple aspects of tumor biology. Genes encoding RhoGAPs (ARHGAP), which switch off Rho-like GTPases, are responsible for breast cancer biogenesis. However, the identification of suitable and novel biomarkers for precision treatment and prognosis remains challenging. The present study aimed to evaluate the expression of ARHGAP family genes in breast cancer and investigate the survival data using the Oncomine, Kaplan-Meier Plotter, bcGenExMiner and cBioPortal online databases. The results demonstrated low expression of ARHGAP6, 7, 10, 14, 19, 23 and 24 and high expression of ARHGAP9, 11, 15, 18 and 30 in patients with breast cancer compared with that in healthy individuals. The survival analysis revealed that low expression levels of ARHGAP6, 7 and 19 were associated with poor relapse-free survival (RFS) and overall survival (OS), whereas high expression levels of ARHGAP9, 15 and 30 were associated with preferable RFS and OS. Metastatic relapse data demonstrated that higher expression of ARHGAP9, 15, 19, 25 and 30 were associated with better prognosis and increased expression of ARHGAP11A and 14 exerted negative effects on patient prognosis. The overlapping genes ARHGAP9, 15, 19 and 30 obtained from these bioinformatics analysis tools exhibited significant association with clinical parameters including age, the presence of estrogen receptor, progesterone receptor and epidermal growth factor receptor-2, Scarff-Bloom-Richardson grade and Nottingham prognostic index. In conclusion, bioinformatics analysis revealed that ARHGAP9, 15, 19 and 30, but not other ARHGAP family genes may be promising targets with prognostic value and biological function for precision treatment of breast cancer.

Introduction

Breast cancer is the most common malignant tumor and a leading cause of cancer-related death among females worldwide. Cases in China account for 12.2% of newly diagnosed breast cancers and 9.6% of breast cancer-associated deaths worldwide in 2014 (1). Although advances have been achieved in early diagnosis and systemic therapy, the prognosis of patients with breast cancer remains poor. The identification of new sensitive and specific biomarkers for the prognosis of patients with breast cancer is therefore urgently needed.

Rho/Rac/cell division cycle 42-like (Rho-like) GTPases are key regulators of multiple cell functions, including cell polarity control, membrane transport, transcriptional regulation, survival, adhesion and proliferation (2,3). Rho-like GTPases are inactive in GDP-bound form and active in GTP-bound form. GTPase activating proteins (RhoGAPs) are negative regulators of Rho-like GTPases, which exert their functions by catalyzing the conversion of the active GTP-bound state to the inactive GDP-bound state. A family of genes encoding RhoGAPs (ARHGAP) switch off Rho-like GTPases. Genetic alterations of ARHGAP family genes are responsible for cancer biogenesis through the dysregulation of Rho-like GTPases (2,3). Low expression of ARHGAP7 is associated with poor prognosis in patients with estrogen receptor (ER)-positive breast cancer with further decrease in survival in patients with metastatic lesions (4). ARHGAP15 is an androgen-induced gene and has anti-tumor roles associated with the Rac1 pathway (5). ARHGAP18 expression is associated with improved patient outcomes in invasive breast cancer (6). Thus, researchers and clinicians are increasingly considering ARHGAP expression levels as a source of important clinical and predictive therapeutic information.

Although previous studies have reported a general expression profile of ARHGAP family genes in breast cancer,
several challenges remain in the identification of suitable and novel biomarkers for precision treatment and prognosis. The present study aimed to perform bioinformatics analysis of the clinicopathological parameters and survival data associated with ARHGAP family genes in patients with breast cancer by pooling and analyzing several large online databases.

Materials and methods

Oncomine. Oncomine (http://www.oncomine.org) is an online database that incorporates 715 datasets and 86,733 samples and aims to compute gene expression signatures and extract biological insights from the data for cancer research (7). All the mentioned ARHGAP genes were queried in the database and the results were filtered by selecting ‘breast cancer’ and ‘cancer’ vs. ‘normal’ analysis with the threshold of fold change ≥ 2, P ≤ 1x10⁻⁴, and gene rank ≥ top 10%.

Kaplan-Meier plotter. The Kaplan-Meier Plotter (http://kmplot.com/analysis/) provides a powerful platform for assessing the biological relationships between gene expression levels and survival information including relapse-free survival (RFS) and overall survival (OS) in patients with breast cancer (8). P-values, hazard ratios and 95% confidence intervals according to the mRNA expression level (low or high) of each ARHGAP gene were obtained.

bcGenExMiner. The Breast Cancer Gene-Expression Miner v4.1 (bcGenExMiner v4.1; http://bcgenex.centregauducheau.fr/BC-GEM) is a mining tool of published annotated genomics data (9,10). The selected ARHGAP family genes were analyzed with clinical parameters such as age, nodal status, the presence of estrogen receptor (ER), progesterone receptor (PR) and epidermal growth factor receptor-2 (HER-2), Scarff-Bloom-Richardson (SBR) grade and Nottingham prognostic index (NPI). Prognostic values of metastatic relapse event and ARHGAP genes were calculated using the prognostic module (9,10).

cBioPortal. The cBioPortal (http://www.cbioportal.org) database offers visualization, analysis and download of large-scale cancer genomics datasets (11,12). To analyze the ARHGAP-centered regulation system, a network of the ARHGAP family and the neighboring genes was generated in cBioPortal.

Statistical analysis. According to protocols of the aforementioned tools, mRNA levels of ARHGAP in breast cancer and normal tissues in each individual dataset were analyzed using Student's t-test. Kaplan-Meier survival analysis was performed to compare patient survival based on ARHGAP expression levels by log-rank test. Global significant difference between groups of clinical parameters was assessed by Welch's and Dunnett-Tukey-Kramer's tests. Data are presented as the mean ± standard error of mean (SEM). P<0.05 was considered to indicate a statistically significant difference.

Results

Dysregulated expression of ARHGAP genes in patients with breast cancer. The expression of ARHGAP family genes in were evaluated in 20 common types of cancer, and their levels were compared to normal individuals using the Oncomine database. Lower expression levels (blue) of ARHGAP6, 7, 10, 14, 19, 23 and 24 and higher expression levels (red) of ARHGAP9, 11, 15, 18 and 30 were observed in breast cancer samples compared with normal tissues. ARHGAP4, 8, 25 and 29 were neither upregulated nor downregulated in patients with breast cancer compared with healthy individuals (Fig. 1).

ARHGAP genes and clinicopathological characteristics of patients with breast cancer. By comparing the aforementioned databases, the expression levels of the overlapped genes ARHGAP9, 15, 19 and 30 were analyzed between different patient groups based on clinicopathological characteristics. The SBR grade, which evaluates tubule formation, nuclear characteristics of pleomorphism and mitotic index, is an important prognostic factor in breast cancer (13). Patients with high grade (SBR3) tumors tended to express high levels of ARHGAP9 and 30 and low levels of ARHGAP19 than lower grade (SBR1) tumors (Fig. 4A). The NPI is based on histopathological factors and is used to stratify patients with breast cancer into prognostic groups (14); low expression of ARHGAP19 was associated with NPI (Fig. 4B). No significant differences were observed between the ≤51 and >51 years groups, with an exception for ARHGAP19, which was expressed at low levels in the >51 years group. Patients with ER-positive or PR-positive breast cancer exhibited lower expression levels of ARHGAP9, 15 and 30 compared with patients with ER-negative or PR-negative status. Patients with HER-2-positive status exhibited higher expression levels of ARHGAP9 and 30 compared with patients with HER-2-negative status. In addition, ARHGAP9, 15, 19 and 30 expression levels were significantly elevated in patients with triple-negative breast cancer compared with patients without triple-negative breast cancer. No significant association was observed between nodal status and ARHGAP9, 15, 19 and 30 expression levels (Fig. 5).

Construction of the ARHGAP gene network. To better visualize the potential genes interacting with ARHGAP9, 15, 19 and 30, a gene network was constructed using the
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Table I. Prognostic association between ARHGAP family gene expression in breast cancer and relapse-free survival based on Kaplan-Meier Plotter analysis.

| Gene name | Cut-off value | Expression (range of probe) | P-value | HR (95% CI) | N |
|-----------|---------------|-----------------------------|---------|-------------|---|
| ARHGAP4   | 125           | 6-1003                      | >0.0001 | 0.75 (0.67-0.83) | 3,951 |
| ARHGAP6   | 136           | 3-2602                      | 0.0003  | 0.82 (0.73-0.91) | 3,951 |
| ARHGAP7   | 901           | 26-10898                    | 0.0350  | 0.89 (0.80-0.99) | 3,951 |
| ARHGAP8   | 867           | 6-8528                      | 0.0004  | 0.82 (0.74-0.91) | 3,951 |
| ARHGAP9   | 398           | 24-4139                     | >0.0001 | 0.60 (0.51-0.70) | 1,764 |
| ARHGAP10  | 271           | 16-1124                     | 0.0015  | 0.84 (0.75-0.94) | 3,951 |
| ARHGAP11A | 45            | 1-806                       | 0.1300  | 1.09 (0.97-1.21) | 3,951 |
| ARHGAP14  | 221           | 6-1787                      | >0.0001 | 0.70 (0.63-0.78) | 3,951 |
| ARHGAP15  | 316           | 4-4586                      | >0.0001 | 0.67 (0.60-0.75) | 3,951 |
| ARHGAP18  | 157           | 3-1189                      | 0.0010  | 0.77 (0.66-0.90) | 1,764 |
| ARHGAP19  | 246           | 12-1183                     | 0.0170  | 0.88 (0.79-0.98) | 3,951 |
| ARHGAP23  | 237           | 9-5617                      | 0.0070  | 0.81 (0.69-0.94) | 1,764 |
| ARHGAP24  | 110           | 3-1758                      | >0.0001 | 0.72 (0.65-0.81) | 3,951 |
| ARHGAP25  | 171           | 3-4021                      | 0.0051  | 0.86 (0.77-0.95) | 3,951 |
| ARHGAP29  | 112           | 1-1992                      | >0.0001 | 0.57 (0.49-0.67) | 1,764 |
| ARHGAP30  | 441           | 21-3316                     | >0.0001 | 0.67 (0.57-0.78) | 1,764 |

ARHGAP, Rho GTPase-activating protein; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 1: Prognostic association between ARHGAP family gene expression in breast cancer and relapse-free survival based on Kaplan-Meier Plotter analysis.

| Cancer type | Cancer vs normal | ARHGAP4 | ARHGAP6 | ARHGAP7 | ARHGAP8 | ARHGAP9 | ARHGAP10 | ARHGAP11A | ARHGAP14 | ARHGAP15 | ARHGAP18 | ARHGAP19 | ARHGAP23 | ARHGAP24 | ARHGAP25 | ARHGAP29 | ARHGAP30 |
|-------------|------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Bladder cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Brain and CNS cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Breast cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Cervical cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Colorectal cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Esophageal cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Gastric cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Head and neck cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Kidney cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Leukemia | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Liver cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Lung cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Lymphoma | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Melanoma | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Other cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Ovarian cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Prostate cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Rectum cancer | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Sarcoma | normal | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Figure 1: Expression of ARHGAP family genes in 20 common types of cancer vs. corresponding normal tissues in the Oncomine database. P≤0.0001, fold change ≥2, gene rank ≥ top 10%. Red and blue represent the numbers of datasets with statistically significantly (P<0.05) upregulated or downregulated ARHGAP family gene mRNA expression, respectively. ARHGAP, Rho GTPase-activating protein.

cBioPortal online database. ARHGAP23 and 39 were connected to ARHGAP9, 15, 19 and 30. Of note, ARHGAP9, 15 and 19 also interacted with ARHGAP30 (Fig. 6).

Discussion

Rho-like GTPases are involved in various cell functions and are negative regulators of Rho proteins; RhoGAPs serve significant roles in multiple aspects of tumor biology including gene expression, cell cycle, survival, migration and invasion (3,6). ARHGAP, a group of family genes encoding RhoGAPs that switch off Rho-like GTPases, have been extensively studied since the discovery that genetic alterations of ARHGAP family genes are responsible for breast cancer biogenesis (2,3). However, several challenges remain regarding the identification of suitable and novel biomarkers for precision treatment and prognosis. To best of our knowledge, this is the first report to characterize specific ARHGAP genes with prognostic value.
and biological function in breast cancer using bioinformatics analysis.

Although the involvement of ARHGAP in cancer progression is becoming increasingly apparent, the association between ARHGAP expression and most cancer types has not been fully characterized. Therefore, a thorough study to determine the expression of ARHGAP in different types of cancer is needed. Researchers and clinicians are increasingly regarding ARHGAP family genes as important clinical and predictive therapeutic information. In the present study, a total of 16 ARHGAP family genes were evaluated in breast cancer samples and compared with those in normal tissues using the Oncomine database; the results demonstrated that ARHGAP6, 7, 10, 14, 19 and 24 exhibited worse RFS compared with patients in the respective high expression groups. (B) Patients with breast cancer with high expression (red) of ARHGAP9, 15 and 30 exhibited better RFS compared with patients in the respective low expression groups. RFS, relapse-free survival; ARHGAP, Rho GTPase-activating protein; HR, hazard ratio.

Figure 2. Kaplan-Meier curves of RFS based on ARHGAP family gene mRNA levels. (A) Patients with breast cancer with low expression (black) of ARHGAP6, 7, 10, 14, 19 and 24 exhibited worse RFS compared with patients in the respective high expression groups. (B) Patients with breast cancer with high expression (red) of ARHGAP9, 15 and 30 exhibited better RFS compared with patients in the respective low expression groups. RFS, relapse-free survival; ARHGAP, Rho GTPase-activating protein; HR, hazard ratio.

exhibited no dysregulation. The number of significant unique analyses was small for ARHGAP6, 9, 10, 14, 15, 23 and 30 or even zero in ARHGAP4, 8, 25 and 29; however, bioinformatics analysis was performed using all genes to identify several suitable and novel biomarkers among ARHGAP family genes. The results of the Kaplan-Meier survival analysis demonstrated that reduced ARHGAP6, 7 and 19 were associated with poor RFS and OS, whereas increased ARHGAP9, 15 and 30 were associated with preferable RFS and OS. In addition, bc-GenExMiner online software provided metastatic relapse data, which revealed that ARHGAP9, 15, 18, 19, 25 and 30 were associated with favorable prognosis, whereas high expression levels of ARHGAP11A and 14 exerted negative effects on patient prognosis. Therefore, ARHGAP9, 15, 19 and 30 were identified as potential prognostic targets for
breast cancer by comparing databases and the overlapped genes. **ARHGAP9**, which is a mitogen-activated protein kinase-docking protein, inhibits mitogen-activated protein kinase 1

Table II. Prognostic association between **ARHGAP** family gene expression in breast cancer and overall survival based on Kaplan-Meier Plotter analysis.

| Gene name  | Cut-off value | Expression (range of probe) | P-value  | HR (95% CI)         | N  |
|------------|---------------|-----------------------------|----------|---------------------|----|
| ARHGAP4    | 120           | 10-699                      | 0.0670   | 0.82 (0.66-1.01)    | 1,402 |
| ARHGAP6    | 157           | 3-3919                      | 0.0150   | 0.77 (0.62-0.95)    | 1,402 |
| ARHGAP7    | 1,031         | 37-10272                    | 0.0100   | 0.76 (0.61-0.94)    | 1,402 |
| ARHGAP8    | 850           | 7-8528                      | 0.6000   | 1.06 (0.85-1.31)    | 1,402 |
| ARHGAP9    | 414           | 41-3927                     | 0.0072   | 0.65 (0.47-0.89)    | 626  |
| ARHGAP10   | 298           | 21-1124                     | 0.6300   | 1.05 (0.85-1.31)    | 1,402 |
| ARHGAP11A  | 46            | 1-806                       | 0.7200   | 1.04 (0.84-1.29)    | 1,402 |
| ARHGAP14   | 216           | 6-1540                      | 0.0550   | 0.81 (0.65-1.00)    | 1,402 |
| ARHGAP15   | 363           | 4-4586                      | >0.0001  | 0.61 (0.49-0.76)    | 1,402 |
| ARHGAP18   | 170           | 5-1189                      | 0.0660   | 0.75 (0.55-1.02)    | 626  |
| ARHGAP19   | 235           | 16-1078                     | 0.0003   | 0.67 (0.54-0.83)    | 1,402 |
| ARHGAP23   | 219           | 9-4750                      | 0.1300   | 1.27 (0.93-1.74)    | 626  |
| ARHGAP24   | 115           | 5-1758                      | 0.4500   | 0.92 (0.74-1.14)    | 1,402 |
| ARHGAP25   | 172           | 7-2335                      | 0.0006   | 0.68 (0.55-0.85)    | 1,402 |
| ARHGAP29   | 126           | 2-1388                      | 0.0250   | 0.70 (0.51-0.96)    | 626  |
| ARHGAP30   | 470           | 21-2542                     | 0.0016   | 0.60 (0.44-0.83)    | 626  |

**ARHGAP**, Rho GTPase-activating protein; HR, hazard ratio; 95% CI, 95% confidence interval.

Figure 3. Kaplan-Meier curves of OS based on **ARHGAP** family gene mRNA levels. (A) Patients with breast cancer with low expression (black) of **ARHGAP6**, 7, 10, 14, 19 and 24 exhibited worse OS compared with patients in the respective high expression groups. (B) Patients with breast cancer with high expression (red) of **ARHGAP9**, 15 and 30 exhibited better OS compared with patients in the respective low expression groups. OS, overall survival; **ARHGAP**, Rho GTPase-activating protein; HR, hazard ratio.
and p38α activation through WW domain binding (15,16). ARHGAP9 has been demonstrated to suppress the migration and invasion of hepatocellular carcinoma cells by upregulating forkhead box J2 and E-cadherin (17). In addition, down-regulated ARHGAP9 is associated with breast cancer risk and suppresses the proliferation, migration and invasion of breast cancer cells (18). The present study also demonstrated that high levels of ARHGAP9 were associated with RFS and OS advantages in patients with breast cancer patients and may be a promising prognostic factor.

ARHGAP15, a Rac-specific RhoGAP described in 2003, serves a dual role in inhibiting small GTPase signaling (19, 20). A previous study has demonstrated that decreased expression of ARHGAP15 promotes the development of colorectal cancer through the PTEN/AKT/forkhead box protein O1 axis (21). In addition, ARHGAP15 is an androgen-induced

| Gene name    | P-value | HR  | 95% CI    | N   | Metastatic relapse |
|--------------|---------|-----|-----------|-----|--------------------|
| ARHGAP4      | 0.4483  | 0.98| 0.92-1.04 | 3,825| 993                |
| ARHGAP6      | 0.4608  | 0.97| 0.89-1.06 | 3,500| 907                |
| ARHGAP7      | 0.1422  | 0.94| 0.87-1.02 | 3,924| 1,023              |
| ARHGAP8      | 0.1410  | 1.09| 0.97-1.21 | 1,345| 340                |
| ARHGAP9      | 0.0244  | 0.89| 0.81-0.99 | 1,721| 438                |
| ARHGAP10     | 0.3605  | 1.03| 0.96-1.11 | 3,456| 878                |
| ARHGAP11A    | 0.0099  | 1.09| 1.02-1.17 | 3,826| 993                |
| ARHGAP14     | <0.0001 | 1.20| 1.11-1.29 | 3,610| 911                |
| ARHGAP15     | <0.0001 | 0.84| 0.79-0.89 | 3,701| 966                |
| ARHGAP18     | <0.0001 | 0.82| 0.75-0.90 | 2,016| 539                |
| ARHGAP19     | 0.0006  | 0.89| 0.84-0.95 | 3,923| 1,023              |
| ARHGAP23     | 0.0574  | 1.15| 1.00-1.33 | 1,425| 358                |
| ARHGAP24     | 0.1126  | 0.94| 0.88-1.01 | 3,845| 1,006              |
| ARHGAP25     | 0.0009  | 0.89| 0.83-0.95 | 3,826| 993                |
| ARHGAP29     | 0.0649  | 0.94| 0.88-1.00 | 3,925| 1,023              |
| ARHGAP30     | 0.0490  | 0.90| 0.81-1.00 | 1,862| 491                |

Table III. Prognostic association ARHGAP family gene expression in breast cancer between and metastatic relapse based on bc-GenExMiner analysis.

ARHGAP, Rho GTPase-activating protein; HR, hazard ratio; 95% CI, 95% confidence interval.

Figure 4. ARHGAP family gene expression in patients with different SBR grades and NPI values. The association between mRNA expression levels of ARHGAP9, 15 and 30 and (A) SBR or (B) NPI value based on the bc-GenExMiner online software. ARHGAP, Rho GTPase-activating protein; SBR, Scarff-Bloom-Richardson grade; NPI, Nottingham prognostic index.
gene and serves an antitumor function associated with the Rac1 pathway (5). These results, along with the results of the survival analysis based on ARHGAP15 expression levels in the present study, suggested that ARHGAP15 may serve as a tumor suppressor during breast cancer progression and metastasis.

Figure 5. The association between mRNA expression of ARHGAP9, 15, 19 and 30 and clinicopathological characteristics of patients with breast cancer based on the bc-GenExMiner online software. ARHGAP, Rho GTPase-activating protein; N, node status; ER, estrogen receptor; PR, progesterone receptor; HER2, epidermal growth factor receptor-2; TNBC, triple-negative breast cancer.
**ARHGAP30**, a Rac1- and RhoA-specific RhoGAP, is a Wrch-1-interacting protein involved in actin dynamics and cell adhesion (22). *ARHGAP30* is required for p53 acetylation and functional activation in colorectal cancer; ectopic expression of *ARHGAP30* induced p53 activation and efficiently suppressed tumor growth in an *in vivo* xenograft study (23). Therefore, *ARHGAP30* may be a prognostic marker and a potential therapeutic target for cancer, which is consistent with the results of the bioinformatics analysis in the present study.

*ARHGAP19* is predominantly expressed in hematopoietic cells and controls cytokinesis and chromosome segregation in T lymphocytes (24). In the present study, low *ARHGAP19* expression levels were associated with poor RFS and OS. In addition, bc-GenExMiner software provided metastatic relapse data, which demonstrated that high *ARHGAP19* expression was associated with favorable metastatic relapse-free survival. These results, along with the observation of decreased *ARHGAP19* expression in patients with high-grade tumors compared with patients with low-grade tumors, opposed the result that *ARHGAP19* expression levels were elevated in patients with triple-negative breast cancer. Since there is limited information on *ARHGAP19* expression in breast cancer, further studies are necessary to determine how *ARHGAP19* is involved in breast cancer biology and progression.

Patients with ER- or PR-positive breast cancer are often treated with drugs that block estrogen effects and generally exhibit good prognosis with compared with ER- or PR-negative patients. The results of the present study demonstrated that *ARHGAP9, 15, 19 and 30* exhibited lower expression in ER- or PR-positive cancer compared with ER- or PR-negative cases, which contradicted the earlier conclusion that *ARHGAP9, 15 and 30* acts as tumor suppressors. This difference may be due to the *ARHGAP* expression profiles in the dataset being primarily from RNA sequences, as well as due to differences in the clinical samples and experimental conditions. Further investigations are required to precisely elucidate the physiological relevance of *ARHGAP9, 15, 19 and 30*.

In conclusion, the results of the present study suggested that *ARHGAP9, 15, 19 and 30*, compared with other *ARHGAP* family genes, might be promising targets with prognostic value and biological function for precision treatment in patients with breast cancer. Further experiments and clinical trials are required to validate the value of these genes.

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Availability of data and materials
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
WXC, ML, LC, YLZ and HD conceived and designed the experiments. WXC, LC and QQ performed the experiments. QQ, LYX and LS analyzed the data. YLZ and HD contributed to reagents, materials and analysis tools. WXC and ML wrote the manuscript. HD gave final approval of the version to be published.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Fan L, Strasser-Weippel K, Li JJ, St Louis J, Finkelstein DM, Yu KD, Chen WQ, Shao ZM and Goss PE: Breast cancer in China. Lancet Oncol 15: e279-e289, 2014.
2. Vega FM and Ridley AJ: Rho GTPases in cancer cell biology. FEBS Lett 582: 2093-2101, 2008.
3. Etienne-Manneville S and Hall A: Rho GTPases in cell biology. Nature 420: 629-635, 2002.
4. Gökmen-Polar Y, True JD, Vieth E, Gu Y, Gu X, Qi GD, Mosley AL and Badve SS: Quantitative phosphoproteomic analysis identifies novel functional pathways of tumor suppressor DLC1 in estrogen receptor positive breast cancer. PLoS One 13: e0204658, 2018.
5. Takagi K, Miki Y, Onodera Y, Ishida T, Watanabe M, Sasano H and Suzuki T: ARHGAP15 in human breast carcinoma: A potent tumor suppressor regulated by androgens. Int J Mol Sci 19: E804, 2018.
6. Aleskandarany MA, Sonbul S, Surridge R, Mukherjee A, Caldas C, Diez-Rodriguez M, Ashankhya I, Albrahim KI, Elmouma AM, Anjela R, et al: Rho-GTPase activating-protein 18: A biomarker associated with good prognosis in invasive breast cancer. Br J Cancer 117: 1176-1184, 2017.
7. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A and Chinnaiyan AM: ONCOMINE: A cancer microarray database and integrated data-mining platform. Neoplasia 6: 1-6, 2004.
8. Györffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q and Szallasi Z: An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 123: 725-731, 2010.
9. Jézéquel P, Frénel JS, Campion L, Guérin-Charbonnel C, Gouraud W, Ricolleau G and Campone M: bc-GenExMiner 3.0: New mining module computes breast cancer gene expression correlation analyses. Database (Oxford) 2013: bas060, 2013.
10. Jézéquel P, Campone M, Gouraud W, Guérin-Charbonnel C, Leux C, Ricolleau G and Campion L: bc-GenExMiner: An easy-to-use online platform for gene prognostic analyses in breast cancer. Breast Cancer Res Treat 131: 765-775, 2012.
11. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioportal. Sci Signal 6: pl1, 2013.
12. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al: The cBio cancer genomics portal: An open platform for exploring multi-dimensional cancer genomics data. Cancer Discov 2: 401-404, 2012.
13. Le Doussal V, Tubiana-Hulin M, Friedman S, Hacene K, Spyromatos F and Brunet M: Prognostic value of histologic grade nuclear components of Scarf-I-Bloom-Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. Cancer 64: 1914-1921, 1989.
14. Haybittle JL, Blamey RW, Elston CW, Johnson J, Doyle PJ, Campbell FC, Nicholson RI and Griffiths K: A prognostic index in primary breast cancer. Br J Cancer 45: 361-366, 1982.
15. Ang BK, Lim CY, Koh SS, Sivakumur N, Taib S, Lim KB, Ahmed S, Rajagopal G and Ong SH: ArgGAP9, a novel MAP kinase docking protein, inhibits Erk and p38 activation through Ww domain binding. J Mol Signal 2: 1, 2007.
16. Furukawa Y, Kawasaki T, Daigo Y, Nishiwaki T, Ishiiho T, Ishihiro K, Takahashi M, Kitayama J and Nakamura Y: Isolation of a novel human gene, ARHGAP9, encoding a rho-GTPase activating protein. Biochem Biophys Res Commun 284: 643-649, 2001.
17. Zhang H, Tang QF, Sun MY, Zhang CY, Zhu JY, Shen YL, Zhao B, Shao ZY, Zhang LJ and Zhang H: ARHGAP9 suppresses the migration and invasion of hepatocellular carcinoma cells through up-regulating FOxJ2/E-cadherin. Cell Death Dis 9: 916, 2018.
18. Wang T and Ha M: Silencing ARHGAP9 correlates with the risk of breast cancer and inhibits the proliferation, migration, and invasion of breast cancer. J Cell Biochem 119: 7747-7756, 2018.
19. Radu M, Rawat SJ, Beesa A, Iliuk A, Tao WA and Chernoff J: ArhGAP15, a Rac-specific GTPase-activating protein, plays a dual role in inhibiting small GTPase signaling. J Biol Chem 288: 21117-21125, 2013.
20. Seoh ML, Ng CH, Yong J, Lim L and Leung T: ArhGAP15, a novel human RacGAP protein with GTPase binding property. FEBS Lett 559: 131-137, 2003.
21. Pan S, Deng Y, Fu J, Zhang Y, Zhang Z, Xu X and Qin X: Decreased expression of ARHGAP15 promotes the development of colorectal cancer through PTEN/AKT/FOXO1 axis. Cell Death Dis 9: 673, 2018.
22. Naji L, Pacholsky D and Aspenström P: ARHGAP30 is a Wrch-1-interacting protein involved in actin dynamics and cell adhesion. Biochem Biophys Res Commun 409: 96-102, 2011.
23. Wang J, Qian J, Hu Y, Kong X, Chen H, Shi Q, Jiang L, Wu C, Zou W, Chen Y, et al: ArhGAP30 promotes p53 acetylation and function in colorectal cancer. Nat Commun 5: 4735, 2014.
24. David MD, Petit D and Bertoglio J: The RhoGAP ARHGAP19 controls cytokinesis and chromosome segregation in T lymphocytes. J Cell Sci 127: 400-410, 2014.

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