Downregulation of Ras GTPase-activating protein 1 is associated with poor survival of breast invasive ductal carcinoma patients

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Abstract. Ras GTPase-activating protein 1 (RASA1) functions to inactivate Ras-GTPase and inhibit the mitogenic signal. Reduction or loss of RASA1 expression occurs during human cancer development and progression. This study investigated RASA1 expression in normal and breast cancer tissue specimens to determine the association with prognosis of breast cancer patients. Two sets of patient samples (45 fresh tissues and 373 paraffin-embedded tissues) were analyzed for RASA1 expression using RT-qPCR and immunohistochemistry. The results showed that the expression of RASA1 mRNA was lower in breast cancer tissues than in the corresponding normal tissues (P<0.001). Additionally, RASA1 expression was reduced in 60.6% (226/373) of breast cancer tissues. The reduced RASA1 expression was significantly associated with tumor lymph node metastasis (P=0.002), advanced TNM stages (P=0.017), estrogen receptor (ER) expression (P=0.002), Ki-67 (P=0.009), higher histological grade (P<0.001), and triple-negative breast cancer (P=0.041). Moreover, the reduced RASA1 expression was associated with shorter disease-free survival (P=0.036) and overall survival (P<0.001) of breast cancer patients. RASA1 expression, together with tumor lymph-node metastasis, TNM stage, Her-2 expression, and triple-negative breast cancer were independent factors in predicting survival of breast cancer patients. In conclusion, RASA1 expression is frequently reduced in breast cancer tissues, and the reduced RASA1 expression is associated with breast cancer progression and poor survival and disease-free survival of patients.

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Introduction

Breast cancer is the most significant worldwide health concern for females, accounting for the leading cause of cancer incidence and the second mortality rate in 2008 (1-3). In spite of advancements in early detection, treatment options, and prevention strategies for controlling breast cancer, a high percentage of breast cancer cases continue to progress, metastasize, and eventually cause patients to succumb to the disease. In this regard, identification of genes or molecular pathways that are responsible for the development and progression of breast cancer with understanding of their clinical significance are necessary, and may aid in the development of novel approaches for the effective treatment and prognostic prediction of breast cancer. Towards this end, our study focused on Ras GTPase-activating protein 1 (RASA1), which functions to inactivate Ras-GTPase and inhibit mitogenic signaling to downstream protein partners through its N-terminal SH2-SH3-SH2 domains (4-6). Aberrant expression of RASA1 protein occurs in ~30% of all human cancers, including breast cancer, but in up to 90% of pancreatic cancers (7).

The Ras family members belong to a class of small GTPase proteins that switch between the active GTP-bound and inactive GDP-bound states to control intracellular signaling networks; for example, the activation of Ras signaling can lead to cell cycle progression and cell proliferation, and can alter cell differentiation, adhesion, and migration, while reducing cell apoptosis (4,5). Altered expression of the Ras oncogene and Ras-related proteins promotes tumorigenesis and increases tumor cell invasion and metastasis (4,5). As a Ras family member, P120 Ras GTPase-activating protein (RasGAP) encoded by the RASA1 gene is involved in the negative regulation of the Ras oncogene (8), with an altered expression of RASA1 protein being reported in several types of carcinoma (9,10). A reduction of RASA1 expression or activity can trigger the RAS-MAPK signaling pathway by activating the GTPase activity of RAS, thereby increasing cell proliferation, suppressing apoptosis, deregulating the cell cycle, and eventually leading to the malignant transformation of cells (10). Thus, in this study, we assessed RASA1 expression in breast cancer tissues and determined whether aberrant expression was associated with clinicopathological factors and

prognosis of breast cancer patients. This allowed us to evaluate the potential of using RASA1 expression as a biomarker for predicting clinical outcome and prognosis of breast invasive ductal carcinoma patients (IDC).

Materials and methods

Patients and tissue specimens. In this study, we collected two sets of tissue specimens. The first set included 45 patients with breast IDC who were subjected to surgical resection for breast cancer at The Third Affiliated Hospital, Harbin Medical University between March and August 2012. We also collected fresh breast cancer tissue samples and corresponding non-tumor breast tissue immediately after surgical resection, which were immediately snap-frozen in liquid nitrogen and stored at -80˚C at the Heilongjiang Breast Tumor Biobank. The second set of breast cancer tissue specimens, 373 paired paraffin-embedded IDC and corresponding non-tumor breast tissue blocks, were obtained from the Department of Pathology, The Third Affiliated Hospital, Harbin Medical University between March and December 2006. The percentage of breast cancer in the tumor specimens was >75%. All the patients were confirmed as breast IDC by a pathological diagnosis of breast tissues and all the samples were collected prior to any radiotherapy or chemotherapy. The Ethics Committee of Harbin Medical University approved our study protocol and written informed consent was obtained from each patient.

Clinicopathological data were collected from patient medical records and the patients (n=373) were followed up until December 2012. The patients were closely monitored after surgery with a median follow-up period of 74 months (range, 3-82 months). Overall survival (OS) and disease-free survival (DFS) were defined as the time from the date of surgery to date of death and the first recurrence, respectively.

Individual samples were routinely assessed for expression of the estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67 by immunohistochemistry. A tumor lesion (case) with ≥14% of Ki-67-positive tumor cells was considered highly proliferative (11). The intensity of anti-HER2 staining was semi-quantitatively analyzed and graded as 0-3: grade 0 or 1, negative; grade 2, weakly positive; and grade 3, positive. Tissues with a weak positive expression (grade 2) were also subjected to duplicate fluorescence *in situ* hybridization (FISH) analysis of the HER-2 gene and scored as negative (<2-fold change) or positive (>2-fold increase) HER-2 gene amplification (12,13). Furthermore, each breast cancer case was subjected to molecular subtyping according to the following criteria: Luminal A type, ER- and/or PR-positive, HER2-negative and Ki-67-positive cells <14%; Luminal B type, (HER2-negative) ER- and/or PR-positive, HER2-negative and Ki-67-positive cells ≥14%, (HER2-positive) ER- and/or PR-positive, with HER2 being overexpressed and/or amplified; HER2 type, where ER and PR were negative and HER2 was overexpressed and/or amplified; or triple-negative breast cancer type where ER, PR, and HER2 were all negative (14). The clinicopathological characteristics of the 373 patients are listed in Table I.

Quantitative RT-PCR (RT-qPCR). Total RNA was isolated from frozen tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Then, 100 ng of each RNA sample was reverse transcribed into cDNA using a High-Capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA, USA) and qPCR was amplified. The relative levels of RASA1 transcripts were normalized to control GAPDH mRNA using the $2^{-\Delta\Delta Ct}$ method. The primer sequences for RASA1 were forward, 5'-CCAACGCCAAAC AATCAG-3' and reverse, 5'-ATTTCCTTGCCATCCACT-3'; and GAPDH forward, 5'-GCCAGCCGAGCCACAT-3' and reverse, 5'-CTTTACCAGAGTTAAAAGCAGCCC-3'. The qPCR amplification was performed in triplicate at 95˚C for 10 min and 40 cycles of 95˚C for 15 sec and 60˚C for 30 sec. Negative controls consisted of distilled H_2O.

Tissue microarray and immunohistochemistry. Immunohistochemical staining was carried out using the two-step plus poly-HRP method as described previously (15). Briefly, tissue blocks were cut for 4 μm sections and placed on poly-L-lysine coated slides. The slides were deparaffinized, dehydrated, immersed in 10 mM sodium citrate buffer (pH 6.0) and pretreated in a microwave oven for 10 min. This was followed by a 10-min rinse with PBS. After blocking with 3% hydrogen peroxide for 10 min at room temperature, the slides were incubated at 4˚C overnight with primary anti-RasGAP antibody (1:150 mouse mAb; Abcam, Cambridge, MA, USA). Afterwards, the slides were stained with the two-step plus poly-HRP anti-mouse IgG detection system (ZSGB-Bio, Beijing, China). After visualization of the reaction with DAB chromogen, the slides were counterstained with hematoxylin and covered with a glycerin gel. For the negative controls, the primary antibody was substituted with PBS to confirm the specificity of the primary antibody.

The relative levels of RasGAP expression were evaluated semi-quantitatively as described in a previous study (16). Briefly, the percentage and intensity of positive anti-RasGAP staining was evaluated and scored in randomly selected 10 high-power fields (magnification, x400) by two investigators in a blinded manner. The percentage of positively stained tumor cells was scored as 0, zero; 1, <10%; 2, 10-50%; and 3, >50%. The staining intensity was scored as 0, no staining; 1, weak (appearing as light yellow); 2, moderate staining (appearing as yellowish-brown); and 3, strong staining (appearing as brown). The staining index (SI) of individual sections was then calculated as: (averaged staining intensity score) x (proportion score). The cut-off value for anti-RasGAP staining was determined by measuring heterogeneity accordingly. An SI score of 4 as the cut-off value was used to distinguish low (<4) vs. high (≥4) RasGAP expression. The cases with discrepancies in the staining scores were re-reviewed by the two pathologists together with a senior pathologist to reach consensus. Ultimately, the staining assessment and allocation of tumors by two investigators were similar with perfect inter-rater reliability.

Statistical analysis. Data were expressed as either real-case numbers or percentages. The difference between groups was analyzed by the χ^2 test or Fisher's exact test. The OS and DFS of the patients were estimated by the Kaplan-Meier survival curve and analyzed by the log-rank test. The potential

Table I. Association of RASA1 expression with clinicopathological characteristics from invasive ductal carcinoma patients (n=373).

Characteristics	n	RASA1		P-value
		Negative	Positive	
Age (years)				
<50	208	132	76	0.203
≥50	165	94	71	
Tumor size (cm)				
T1 (≤2)	138	75	63	0.059
T2-T3 (>2)	235	151	84	
Lymph node metastasis				
Negative	167	83	84	0.002
Positive	206	143	63	
TNM stage				
I/II	255	144	111	0.017
III	118	82	36	
ER				
Negative	159	111	48	0.002
Positive	214	115	99	
PR				
Negative	126	83	43	0.136
Positive	247	143	104	
HER-2				
Negative	240	148	92	0.568
Positive	133	78	55	
Ki-67				
Negative	162	86	76	0.009
Positive	211	140	71	
Histological grade				
G1-G2	237	160	77	<0.001
G3	136	66	70	
Molecular subtype				
Triple-negative	67	48	19	0.041
Others	306	178	128	
Recurrence				
Yes	139	92	47	0.088
No	234	134	100	
Death				
Yes	76	60	16	<0.001
No	297	166	131	

ER, estrogen receptor; PR, progesterone receptor.

factors affecting survival were analyzed by risk ratios (RRs), 95% confidence intervals (CI), and univariate and multivariate regression analyses using the Cox proportional hazards model. Statistical analyses were performed using SPSS, ver. 11.0. (SPSS, Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

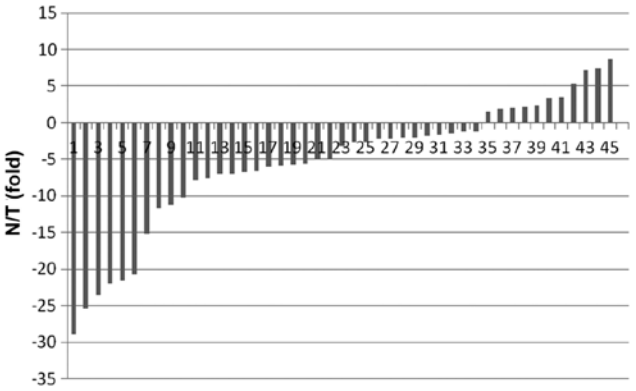

Figure 1. Histogram of RASA1 mRNA expression in 45 invasive ductal carcinoma tissues. Relative expression level of RASA1 mRNA was calculated in each tissue sample as a ratio of N (normal tissue)/T (tumor tissue) or T/N.

Results

Differential expression of RASA1 mRNA and protein in breast IDC tissues. In this study, we first assessed the expression of RASA1 mRNA in 45 samples of breast IDC tissue and found that the mean expression value of RASA1 mRNA in the tumor tissues was 2.93±17.98 (normalized to the GAPDH level), which was significantly lower than that of the corresponding normal tissue (20.16±125.98; P<0.001 by a Wilcoxon rank test). We then defined 2-fold changes as a reduced expression of RASA1 mRNA between the tumor and corresponding normal tissue specimens (Fig. 1). The results revealed that 64.4% (29/45) of breast cancer tissue expressed a low level of RASA1 compared to the paired normal specimens, indicating that the down-regulation of RASA1 expression occurs at the mRNA level. To verify this finding, we used another set of tissue samples to assess RASA1 protein expression and then determined whether the levels of RASA1 expression were associated with clinicopathological data and prognosis of the patients.

We assessed the expression of RASA1 protein in 373 cases of IDC and the paired normal cases using immunohistochemistry. Our results showed that 226 (60.59%) out of 373 breast IDC tissues had a reduced expression of the RasGAP protein, whereas 147 (39.41%) of the 373 breast IDC tissues expressed relatively high levels of RasGAP protein compared to the paired normal breast tissue (Fig. 2). The results regarding the association between RasGAP protein expression and clinicopathological data are shown in Table I. Specifically, the stratification analysis showed that there was no statistically significant difference between RASA1 expression and patient age, tumor size, progesterone receptor (PR) expression, or HER-2 expression. By contrast, the reduced RASA1 expression was significantly associated with lymph-node metastasis (P=0.002), advanced TNM stage (P=0.017), ER expression (P=0.002), Ki-67 expression (P=0.009) and higher histopathological grades (P<0.001). A reduction in RASA1 expression was also frequently observed in triple-negative breast cancers (P=0.041).

Association between RASA1 expression and survival of breast IDC patients. We determined whether levels of RASA1 expression were associated with survival of breast IDC patients

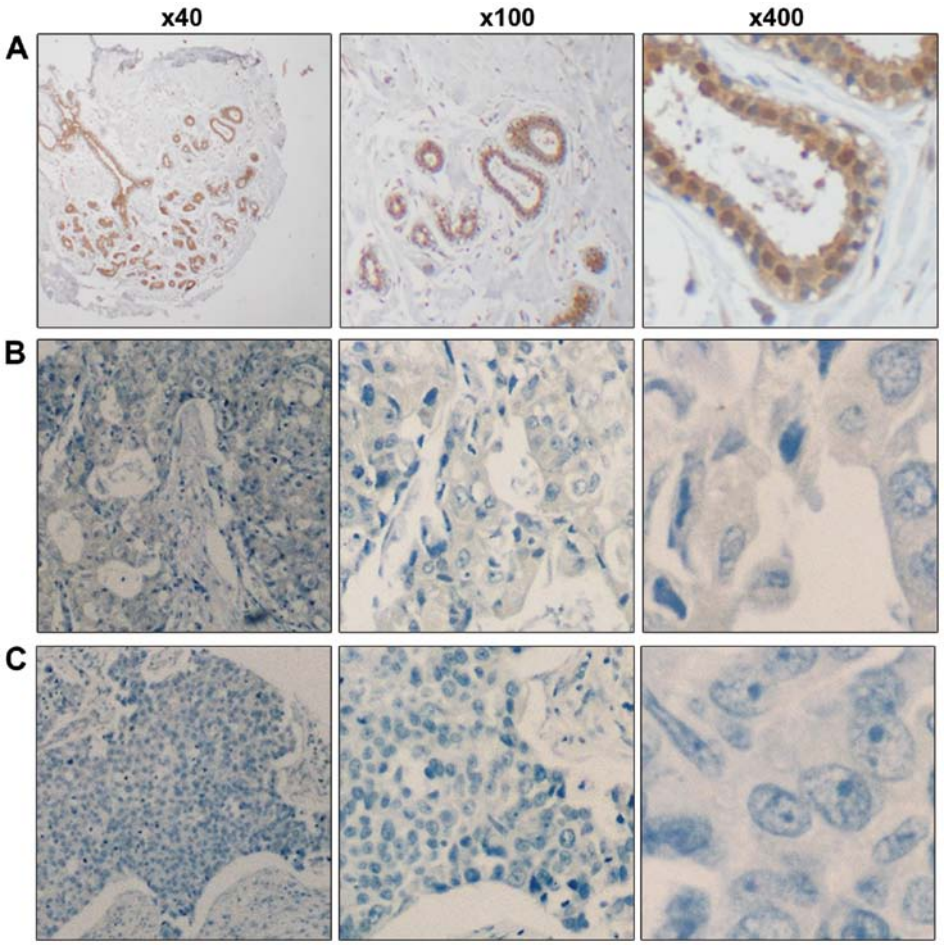

Figure 2. Immunohistochemical staining of RASA1 protein in invasive ductal carcinoma specimens. Representative high expression of RASA1 protein was observed in the nuclei and cytoplasm of normal breast ductal epithelial cells, whereas faintly positive or negative staining was found in tumor cells of breast cancer tissue.

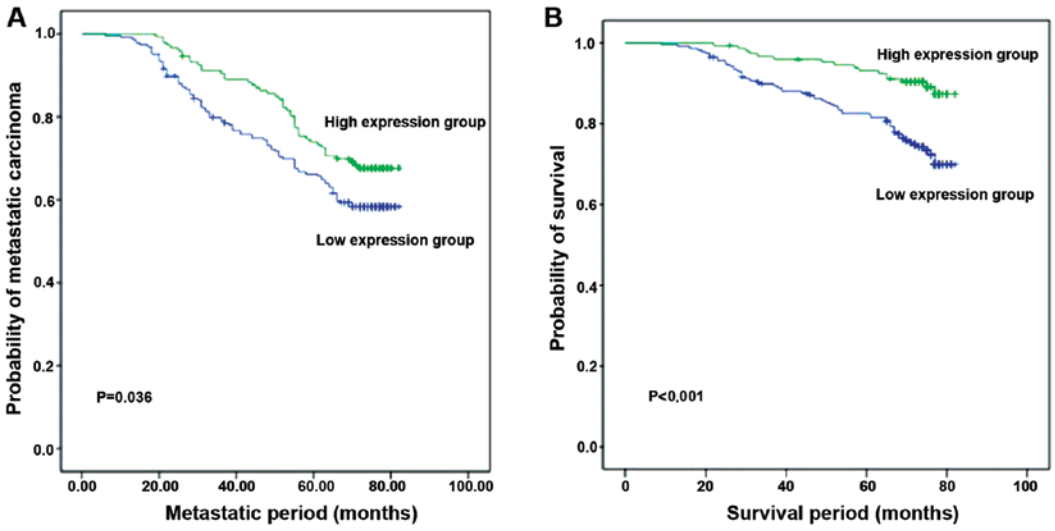

Figure 3. Association of RASA1 expression with overall (OS) and disease-free (DFS) survival of IDC patients. Kaplan-Meier curves of RASA1 expression to determine the association with (A) OS and (B) DFS of 373 invasive ductal carcinoma patients. Patients with the reduced RASA1 expressed tumor had a significantly shorter OS and DFS than those with high RASA1-expressed tumors (P<0.05, log-rank test).

(Fig. 3 and Table II). All of the 373 patients were followed up after surgery with a median of 74 months of the follow-up (ranging between 3 and 82 months). Our results showed that a reduced RASA1 expression was significantly associated with poorer DFS compared to when tumors show high levels of RASA1 expression (P=0.036). Similarly, patients with low

Table II. Univariate and multivariate analyses for overall survival in 373 invasive ductal carcinoma patients.

Characteristics	Univariate analysis			Multivariate analysis		
	RR	95% CI	P-value	RR	95% CI	P-value
Age (≤50/>50 years)	1.281	0.82-2.01	0.281	-	-	-
Tumor size (T1/T2+T3)	1.604	0.97-2.65	0.036	-	-	-
Lymph node metastasis (-/+)	4.625	2.54-8.41	<0.001	2.540	1.23-5.22	0.011
TNM stage (I-II/III)	4.140	2.61-6.57	<0.001	2.365	1.36-4.10	0.002
ER (-/+)	0.335	0.21-0.54	<0.001	-	-	0.188
PR (-/+)	0.376	0.24-0.59	<0.001	-	-	0.727
Her-2 (-/+)	1.664	1.06-2.61	0.027	3.796	2.07-6.96	<0.001
Ki-67	0.639	0.41-1.00	0.051	-	-	-
Histopathological grade (1+2/3)	0.795	0.49-1.29	0.353	-	-	-
Molecular subtype (triple-negative/others)	1.470	1.25-1.73	<0.001	1.806	1.46-2.24	<0.001
RASA1 expression (high/low)	0.362	0.21-0.63	<0.001	0.485	0.28-0.85	0.012

RR, risk ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

levels of RASA1 expression in tumors had a shorter OS rate than patients with tumors showing a high RASA1 expression (P<0.001).

Univariate analysis revealed that lymph-node metastasis (P<0.001), advanced TNM stage (P<0.001), expression of ER (P<0.001) and Her-2 (P=0.027), triple-negative breast cancer (P<0.001) and reduced RASA1 expression (P<0.001), all contributed to poor patient survival. In addition, multivariate analysis showed that lymph node metastasis (P=0.011), advanced TNM stage (P=0.002), Her-2 expression (P<0.001), triple-negative breast cancer (P<0.001) and reduced RASA1 expression (P=0.012) were all independent factors in predicting the survival of these breast cancer patients.

Discussion

Breast cancer is the most frequently diagnosed malignancy in women in western countries and is the most prevalent malignant disease in almost all countries (17). Hampering the development of effective treatment methods, breast cancer is a complex and heterogeneous disease (18). On a molecular level, breast cancer can be classified using ER, PR and Her-2 expression as either ER- and PR-positive or as negative for all three markers (i.e., triple-negative breast cancer). Hormone blocking therapy can effectively control and treat ER- and PR-positive tumors, however, triple-negative breast cancers prove a greater challenge (19,20). Thus, development of novel strategies for the treatment and prognostic prediction of breast cancer, especially triple-negative breast cancer, may aid in the reduction of breast cancer mortality. For example, identification of genes in breast cancer may be used as novel adjuvant diagnostic and prognostic biomarkers to improve treatment decisions in combination with these parameters (21).

In this study, we have demonstrated that the expression of RASA1 is significantly reduced in breast IDC tissues compared to the corresponding normal tissues. Functionally, the *RASA1* gene encodes a p120-RasGTPase-activating protein, which switches the active GTP-bound Ras to the inactive GDP-bound form. In *RASA1* knockout mice, the embryos exhibit abnormal vascular development (22,23). Subsequent studies have demonstrated that RASA1 is an important member of the RAS pathway and a putative tumor suppressor (24). On a molecular level, the p120-RasGAP protein contains two SH2, SH3, PH (pleckstrin homology) and CaLB/C2 (calcium-dependent phospholipid-binding domain) domains at the N-terminal, which function to regulate cell proliferation, migration and apoptosis depending on their downstream binding partners (25-29). The p120-RasGAP protein interacts with other proteins such as Ras, Akt, Aurora and RhoGAP to regulate cell functions in angiogenesis and cancer development. For example, RASA1 acts as a suppressor of RAS function by enhancing the weak intrinsic GTPase activity of RAS proteins (24), resulting in an increase in the inactive GDP-bound form of RAS, thereby leading to aberrant intracellular signaling such as inhibition of the RAF-MEKERK and PI3K-Akt pathways (30-32). In addition, RASA1 is suggested to play a role in the regulation of angiogenesis and tumor progression (33). Our current *ex vivo* experiments have demonstrated that the reduced RASA1 expression was significantly associated with lymph node metastasis, advanced TNM stage, ER expression, Ki-67 expression, higher histopathological grades and triple-negative breast cancers. These findings indicate that a reduced RASA1 expression is likely to contribute to breast cancer progression. A previous study also showed that a reduction in RASA1 expression induced human colorectal cancer cell growth *in vitro* and stimulated tumorigenesis in nude mice (24). Another recent study has shown that the selective suppression of RASA1 promoted the unrestrained activation of Ras signaling in wild-type RAS-expressed hepatocellular carcinoma cells, resulting in increased tumor cell proliferation and resistance to apoptosis (34). Our current study did not assess the cause of the reduced RASA1 expression in breast cancer. However, a previous study identified alterations in chromosome copy number in association with different subtypes, chromosomes that contain different candidate oncogenes and tumor suppressors including RASA1 (9). This suggests that our findings of a

reduced RASA1 expression in breast cancer may occur at the genomic level. Future studies are needed to establish whether the loss of heterozygosity or RASA1 mutations contribute to a reduced RASA1 expression in breast cancer.

Our current study has shown that a low RASA1 expression frequently occurs in triple-negative breast cancers, which is a novel finding. A previous study demonstrated that triple-negative breast cancer was associated with a higher expression of Ki-67 (21). Future studies are needed to confirm our current finding and to investigate whether targeting RASA1 is a novel therapeutic strategy for controlling triple-negative breast cancer. In addition, this study revealed that a reduced RASA1 expression is associated with poor OS and DFS of breast cancer patients, which has not been previously reported. We have also found that RASA1 expression, together with tumor lymph node metastasis, TNM stage, Her-2 expression, and triple-negative breast cancer were all independent factors in predicting the survival of breast cancer patients.

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References

1. Al-Mubarak M, Sacher AG, Ocana A, Vera-Badillo F, Seruga B and Amir E: Fulvestrant for advanced breast cancer: a meta-analysis. Cancer Treat Rev 39: 753-758, 2013.
2. Lianidou ES, Mavroudis D and Georgoulias V: Clinical challenges in the molecular characterization of circulating tumour cells in breast cancer. Br J Cancer 108: 2426-2432, 2013.
3. Lavigne E, Holowaty EJ, Pan SY, *et al*: Breast cancer detection and survival among women with cosmetic breast implants: systematic review and meta-analysis of observational studies. BMJ 346: Apr 29, 2013 (Epub ahead of print). doi: 10.1136/bmj.f2399.
4. Stites EC and Ravichandran KS: A systems perspective of ras signaling in cancer. Clin Cancer Res 15: 1510-1513, 2009.
5. Saxena N, Lahiri SS, Hambarde S and Tripathi RP: RAS: target for cancer therapy. Cancer Invest 26: 948-955, 2008.
6. Liu YF, Deth RC and Devys D: SH3 domain-dependent association of huntingtin with epidermal growth factor receptor signaling complexes. J Biol Chem 272: 8121-8124, 1997.
7. Vigil D, Cherfils J, Rossman KL and Der CJ: Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? Nat Rev Cancer 10: 842-857, 2010.
8. Pamonsinlapatham P, Hadj-Slimane R, Lepelletier Y, *et al*: p120-Ras GTPase activating protein (RasGAP): a multi-interacting protein in downstream signaling. Biochimie 91: 320-328, 2009.
9. Hu X, Stern HM, Ge L, *et al*: Genetic alterations and oncogenic pathways associated with breast cancer subtypes. Mol Cancer Res 7: 511-522, 2009.
10. Fang JY and Richardson BC: The MAPK signalling pathways and colorectal cancer. Lancet Oncol 6: 322-327, 2005.
11. Cheang MC, Chia SK, Voduc D, *et al*: Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 101: 736-750, 2009.
12. Aksoy S, Dizdar O, Harputluoglu H and Altundag K: Demographic, clinical, and pathological characteristics of Turkish triple-negative breast cancer patients: single center experience. Ann Oncol 18: 1904-1906, 2007.
13. Bauer KR, Brown M, Cress RD, Parise CA and Caggiano V: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 109: 1721-1728, 2007.
14. Xue C, Wang X, Peng R, *et al*: Distribution, clinicopathologic features and survival of breast cancer subtypes in Southern China. Cancer Sci 103: 1679-1687, 2012.
15. Zhang Y, Cheng S, Zhang G, *et al*: Low expression of RECK indicates a shorter survival for patients with invasive breast cancer. Cancer Sci 103: 1084-1089, 2012.
16. Liu T, Zhang X, Shang M, *et al*: Dysregulated expression of Slug, vimentin, and E-cadherin correlates with poor clinical outcome in patients with basal-like breast cancer. J Surg Oncol 107: 188-194, 2013.
17. Adam Maciejczyk A: New prognostic factors in breast cancer. Adv Clin Exp Med 22: 5-15, 2013.
18. do Carmo França-Botelho A, Ferreira MC, França JL, França EL and Honorio-França AC: Breastfeeding and its relationship with reduction of breast cancer: a review. Asian Pac J Cancer Prev 13: 5327-5332, 2012.
19. Liedtke C, Mazouni C, Hess KR, *et al*: Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 26: 1275-1281, 2008.
20. Carey LA, Dees EC, Sawyer L, *et al*: The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 13: 2329-2334, 2007.
21. Cadoo KA, McArdle O, O'Shea AM, Power CP and Hennessy BT: Management of unusual histological types of breast cancer. Oncologist 17: 1135-1145, 2012.
22. Henkemeyer M, Rossi DJ, Holmyard DP, *et al*: Vascular system defects and neuronal apoptosis in mice lacking ras GTPase-activating protein. Nature 377: 695-701, 1995.
23. Lapinski PE, Bauler TJ, Brown EJ, Hughes ED, Saunders TL and King PD: Generation of mice with a conditional allele of the p120 Ras GTPase-activating protein. Genesis 45: 762-767, 2007.
24. Sun D, Yu F, Ma Y, *et al*: MicroRNA-31 activates the RAS pathway and functions as an oncogenic MicroRNA in human colorectal cancer by repressing RAS p21 GTPase activating protein 1 (RASA1). J Biol Chem 288: 9508-9518, 2013.
25. Trahey M, Wong G, Halenbeck R, *et al*: Molecular cloning of two types of GAP complementary DNA from human placenta. Science 242: 1697-1700, 1988.
26. Moran MF, Koch CA, Anderson D, *et al*: Src homology region 2 domains direct protein-protein interactions in signal transduction. Proc Natl Acad Sci USA 87: 8622-8626, 1990.
27. Musacchio A, Gibson T, Rice P, Thompson J and Saraste M: The PH domain: a common piece in the structural patchwork of signalling proteins. Trends Biochem Sci 18: 343-348, 1993.
28. Clark JD, Lin LL, Kriz RW, *et al*: A novel arachidonic acid-selective cytosolic PLA2 contains a Ca(2+)-dependent translocation domain with homology to PKC and GAP. Cell 65: 1043-1051, 1991.
29. Gawler DJ, Zhang LJ, Reedijk M, Tung PS and Moran MF: CaLB: a 43 amino acid calcium-dependent membrane/phospholipid binding domain in p120 Ras GTPase-activating protein. Oncogene 10: 817-825, 1995.
30. Glennon TM, Villa J and Warshel A: How does GAP catalyze the GTPase reaction of Ras? A computer simulation study. Biochemistry 39: 9641-9651, 2000.
31. Shurki A and Warshel A: Why does the Ras switch 'break' by oncogenic mutations? Proteins 55: 1-10, 2004.
32. Denhardt DT: Signal-transducing protein phosphorylation cascades mediated by Ras/Rho proteins in the mammalian cell: the potential for multiplex signalling. Biochem J 318: 729-747, 1996.
33. Cailliau K, Browaeys-Poly E and Vilain JP: RasGAP is involved in signal transduction triggered by FGF1 in *Xenopus oocytes* expressing FGFR1. FEBS Lett 496: 161-165, 2001.
34. Calvisi DF, Ladu S, Conner EA, *et al*: Inactivation of Ras GTPase-activating proteins promotes unrestrained activity of wild-type Ras in human liver cancer. J Hepatol 54: 311-319, 2011.