Expression of Signal-induced Proliferation-associated Gene 1 (*SIPA1*), a RapGTPase-activating Protein, Is Increased in Colorectal Cancer and Has Diverse Effects on Functions of Colorectal Cancer Cells

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Abstract. *Background: Signal-induced proliferation-associated gene 1 (SIPA1) codes for a GTPase-activating protein, known to be a negative regulator of Ras-related Protein (RAP) which belongs to the Ras superfamily. It has been implicated in certain malignancies, including leukemia, cervical cancer and breast cancer. However the role of this molecule in colorectal cancer remains unknown. The current study aimed to investigate the expression of SIPA1 in colorectal tumour tissues and its impact on the function of colorectal cancer cells. Materials and Methods: A total of 94 colorectal cancer tissues together with 80 normal background tissues were used to examine the expression of SIPA1 transcript and protein using real-time quantitative Polymerase Chain Reaction (PCR) and immunohistochemical methods, respectively. Any association with clinical and histopathological characteristics was then identified. Ribozyme transgenes targeting SIPA1 were prepared to knockdown the expression of SIPA1 in colorectal cancer cells. The impact on their functions was subsequently determined, using respective in vitro function assays. Results: An increased expression of SIPA1 was evident in colorectal cancer tissues compared with its expression in normal background tissues (p<0.001). In colorectal tumours, its expression appeared to be lower in poorly-differentiated samples and in patients who had lymphatic metastasis. Knockdown of SIPA1 in colorectal cancer cells resulted in reduced cell growth in vitro. The knockdown exhibited a contrasting effect on invasion and migration, both of which were increased in SIPA1-knockdown cells compared with the controls. Conclusion: SIPA1 is up-regulated in colorectal cancer. This suggests that SIPA1 plays diverse roles during disease progression as has contrasting effects on growth and motility of colorectal cancer cells.*

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Despite improvement in screening and prevention, colorectal cancer remains a significant source of morbidity and mortality in Europe and North America, accounting for a remarkable loss of lifes each year (1, 2). Research involving inbred transgenic mice suggests that metastatic probability is potentially related to the genetic variation located in the metastasis-efficiency modifier locus (3). Evidence from sequence analysis, as well as *in vitro* and *in vivo* experiments have all indicated that signal-induced proliferation-associated gene 1 (*Sipa1*) is a strong candidate gene, located at the *Mtes1* locus. Genetic diversity in *SIPA1* has been shown to be involved in the progression and metastasis of breast cancer, particularly being related to the aggressive characteristics of breast cancer cells (4). In humans, *SIPA1*, is localized at 11q13. There are three normal single-nucleotide polymorphisms (SNPs) identified within regulatory or coding regions of *SIPA1* (5). One is an A-to-G SNP (rs931127) located in the promoter region; rs3741378 is a C-to-T SNP that encodes for the replacement of a serine-to-phenylalanine amino acid in exon 3; rs746429 is a G-to-A SNP that encodes for a synonymous amino acid transformation in exon14 (4). During an analysis of 300 cases, the involvement of lymph nodes and lack of hormone receptor in tumours was associated with the variant alleles of these SNPs after age-adjustment (6). It has been proposed that *SIPA1* possibly contributes to the aggressive behavior of cancer cells, with a potential implication for lymph node involvement (7-8).

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Table I. *Primer sequences*.

Gene	Sense primer	Antisense primer
SIPA1	5'-AGAACTCGCTGTCACCAC	5'-ACTGAACCTGACCGTACATACTGATGGCTTGGCTGT
GAPDH	5'-ATGATATCGCCGCGCTCGTC	5'-CTGCAGTGCAGGGGGCACAGGTGGCTGATGAGTCCGTGAGGA
SIPA1 ribozyme	5'-ACTGAACCTGACCGTACACGC TCGGTGAGGATCTTCA	5'-ACTAGTGAGCTAGGCCTGGGTGGACCAGCATTTCGTCCTCACGG -

The product of *SIPA1* gene is a mitogen-induced GTPase-activating protein (GAP), with shows specific GAP activities on Ras-related Protein 1 (RAP1) and Ras-related Protein 2 (RAP2). The RAP family is a subgroup of small GTPases making up the part of Ras superfamily. RAP1 has been indicated to be up-regualted in some types of solid tumours, and an increased expression of RAP1 is associated with invasion and migration of prostate cancer cells (9). As a negative regulator of RAP1 and RAP2, aberrant expression of SIPA1 has also been indicated in leukemia, cervical cancer and breast cancer (11-13). However, the role played by this particular GAP in colorectal cancer remains unknown. The present study aimed to examine its expression in colorectal cancer tissues and discover its impact on the function of human colorectal cancer cells.

Materials and Methods

Colorectal tissues and cell lines. Colorectal cancer tissues (n=94) and normal background tissues (n=80) were collected immediately after surgery from patients with colorectal cancer (with the local Research Ethic Committee approval) and stored at –80˚C until use. Two human colorectal adenocarcinoma cell lines, HT115 and Caco-2 were obtained from the European Collection for Animal Cell Culture (ECACC, Porton Down, Salisbury, UK).

RNA extraction. RNA extraction kits, reverse transcription kits and Reverse Transcription Polymerase Chain Reation Mix were purchased from Promega (WI, USA) and Bio-Rad (CA, USA). Conventional PCR primers were designed using Beacon Designer (PREMIER Biosoft) and synthesized by Invitrogen (Paisley, Scotland, UK). Following the manufacturer's protocol, total RNA was isolated using a standard guanidine isothiocyanate method. The concentration of RNA was determined using UV spectrophotometry at 260 and 280 nm. cDNA samples were synthesized in a total volume of 20 μl of reaction mixture. Glyceraldehyde 3-phosphate Dehydrogenase (GAPDH) primers were used as house keeping genes.

Real-time quantitative polymerase chain reaction (QPCR). Real-time quantitative PCR was used for verifying the level of mRNA expression of *SIPA1* from the prepared cDNA samples. All colorectal cDNA samples were synchronously examined for *SIPA1* along with an applicable set of plasmid standards. QPCR primers for *SIPA1* were designed using the Beacon Design software (PREMIER Biosoft). Sequences of primers used in the current study are provided in Table I. Real-time PCR was carried out using IcyclerIQ™ (Bio-Rad, Hemel Hempstead, UK), following the cycling conditions: 94˚C for 5 min, 80-90 cycles of: 94˚C for 10 s, 55˚C for 35 s and 72˚C for 20 s.

Ribozyme transgene targeting human SIPA1. Anti-human *SIPA1* hammerhead ribozymes were designed using the Zuker NA mFold program, on the basis of the secondary structure of *SIPA1* mRNA (14). The ribozymes were synthesized using touchdown PCR and cloned into the pEF6/V5-His TOPO TA Expression plasmid vector (Invitrogen), according to the protocol provided. Ribozyme transgenes and empty plasmids were transfected into the two colorectal cell lines HT115 and Caco-2, respectively, utilizing an Easyjet Plus electroporator (EquiBio, Kent, UK). Following selection of cells using blasticidin, verified transfectants which had lost the expression of *SIPA1* were used in the subsegment experiments.

In vitro cell growth assay. Cells were seeded into a 96-well plate at 2,500 cells/well, which were cultured using normal media (10% fetal cattle serum, 0.1% antibiotics). The cells were cultured in triplicate for 1, 3 and 5 days. After incubation the cells were fixed in 4% formalin and stained by 0.5% crystal violet (w/v). The stained crystal violet was then extracted using 10% (v/v) acetic acid, and the absorbance was determined using a spectrophotometer (Bio-Tek, ELx800), at a wavelength of 540 nm.

In vitro cell adhesion assay. A 96-well plate was pre-coated with 5 μg of Matrigel (CollaborativeResearch Products, Bedford, MA, USA) and allowed to air dry. Following rehydration using serum-free media, 40,000 cells were seeded into each well. After 40 min of incubation, non-adherent cells were washed-off using balanced salt solution. The adherent cells were then fixed with 4% formalin and stained using 0.5% crystal violet. The number of adherent cells was counted under a microscope.

In vitro invasion assay. Transwell inserts (with 8-μm pores) were pre-coated with 50 μg of Matrigel and air dried. Following rehydration, 40,000 cells were seeded into each insert. After incubation for 3 days, cells which had invaded through the matrix and adhered to the other side of the insert were fixed in 4% formalin, and stained with 0.5% (w/v) crystal violet. The number of invaded cells was then counted under a microscope.

In vitro wounding assay. Cells were seeded into a 24-well plate at a density of 200,000 per well and allowed to form a monolayer of cells. The monolayer of cells was then scraped to create a wound. Migration of the cells at wounding edges was monitored over a period of up to 18 hours. Optimas 6.0 Motion Analysis (Meyer

Instruments, Houston, TX, USA) was used to track the leading edge of the cells, in order to measure the distance of the migration.

Immunohistochemical staining of SIPA1 protein. The frozen sections of colorectal tumors and adjacent background tissues were sectioned at a thickness of 6 μm using a cryostat (15). The samples were mounted onto Super Frost Plus microscope slides. After air-drying, the samples were fixed in a mixture of 50% acetone and 50% methanol and then air-dried once again. After rehydration and blocking with 0.6% solution of horse serum, the sections were probed with a rabbit polyclonal anti-SIPA1 antibody (Santa Cruz Biotechnology, Inc., CA, USA) and subsequently a peroxidase- conjugated anti-rabbit antibody (Sigma, Dorset, UK). Following the instructions, the avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA) was applied before staining with diaminobenzidine chromogenI. Nuclei were counterstained in Gill's haematoxylin.

Data analysis. The relationship between *SIPA1* expression and tumor grade, TNM staging and nodal status was respectively analysed using the Mann-Whitney *U* and Kruskal-Wallis tests. Survival analysis curves were drawn using Kaplan Meier survival analysis. Quantitative data were analysed using the Student's *t*-test, and chi-square test, where appropriate. Differences were considered to be statistically significant at $p<0.05$.

Results

SIPA1 expression in colorectal adenocarcinoma tissues. Transcript levels of *SIPA1* were determined in the colorectal adenocarcinoma samples using quantitative real-time PCR. Increased levels of *SIPA1* were shown in the tumours ($p<0.01$), in comparison with matched background tissues (Table II). Immunohistochemical staining of SIPA1 in the human colorectal adenocarcinoma tissues demonstrated the presence of this protein in the cytoplasm of tumour cells, normal epithelial cells and some stromal cells. More intense staining of SIPA1 was seen in the tumours compared with normal background tissues (Figure 1).

Table II. *SIPA1 transcripts in colorectal carcinomas and correlation with clinical and histopathological parameters.*

Category	No.	Median	IQR	*p*-Value
Normal	80	1.7	0-24	
Tumour	94	132.7	17-382	<0.001
Paired normal	68	1.7	0-24	
Paired tumour	68	163.2	25-418	<0.001
Grade (differentiation)				
Well differentiated	2	175.6	N/A	
Moderately differentiated	54	160.4	17-439	0.912
Poorly differentiated	14	84.7	17.4-340.8	0.475
Tumour stage				
T1	2	161.1	N/A	
T2	10	176	14-2798	0.915
T3	40	133	16-335	0.745
T4	18	149.4	40.6-416.9	0.659
Lymph node involvement				
No	39	163.8	18-465	
N1	16	205.5	2-418	0.810
N2	15	82.1	19.6-211.5	0.247
N1&2	31	95.5	10.8-352.6	0.385
TNM staging				
TNM1	9	162.6	44-2438	
TNM2	30	172.1	17-394	0.617
TNM3	26	88.8	9-344	0.355
TNM4	6	149.4	0-443.7	0.517
Dukes classification				
A	7	223	128-3718	
B	33	164	17-343	0.233
C	32	91.4	4.2-349.7	0.120
Non-invasive	50	149	22-359	
Invasive	26	138	17-417	0.909
Clinical outcome				
Disease-free	35	128	18-495	
Local recurrence	7	158.2	0.5-226.6	0.735
Metastasis	19	103.8	0.5-341	0.541
Death	22	217	86-439	0.294

SIPA1 expression and histopathological characteristics of the disease. We also evaluated the relation of mRNA expression to histopathological features through quantitative analysis of SIPA1 transcript (Table II). The expression of SIPA1 appeared to be lower in poorly-differentiated tumours compared to well-differentiated and moderately-differentiated tumours. No statistical difference was evident between tumours of different sizes. Lower expression levels of SIPA1 were seen in tumours with lymphatic metastases (N2), compared to levels in tumours of lower lymph node involvement (N1), and in tumours without lymph node involvement (N0). Similarly, a decreased expression was seen in tumours categorized as Dukes' C which had lymphatic metastases. In addition to this, analysis according to TNM staging showed that the lowest level of SIPA1 expression was seen in tumours with TNM3, although no statistical significance was seen in these comparisons.

Correlation between SIPA1 expression and clinical outcome. No difference was seen in the expression of SIPA1 in patients who had local recurrence, metastases or who had died from the disease, when compared to that of patients who had remained disease-free at the end of the follow-up period. The average *SIPA1* transcript level of Dukes' B group was used as a threshold. Kaplan–Meier survival analysis showed that patients with a higher expression level of *SIPA1* had lower survival, of 114.7 months (95% CI=87.8-141.6 months), compared to 135.0 months (95% CI=112.6-157.4) for patients with lower expression of SIPA1 (Figure 2).

SIPA1 knock-down on in vitro invasion, adhesion, migration and growth of colorectal cancer cell lines. To examine the function of SIPA1 in colorectal cancer cells, we established *SIPA1*-knockdown sublines of two colorectal cancer cell lines,

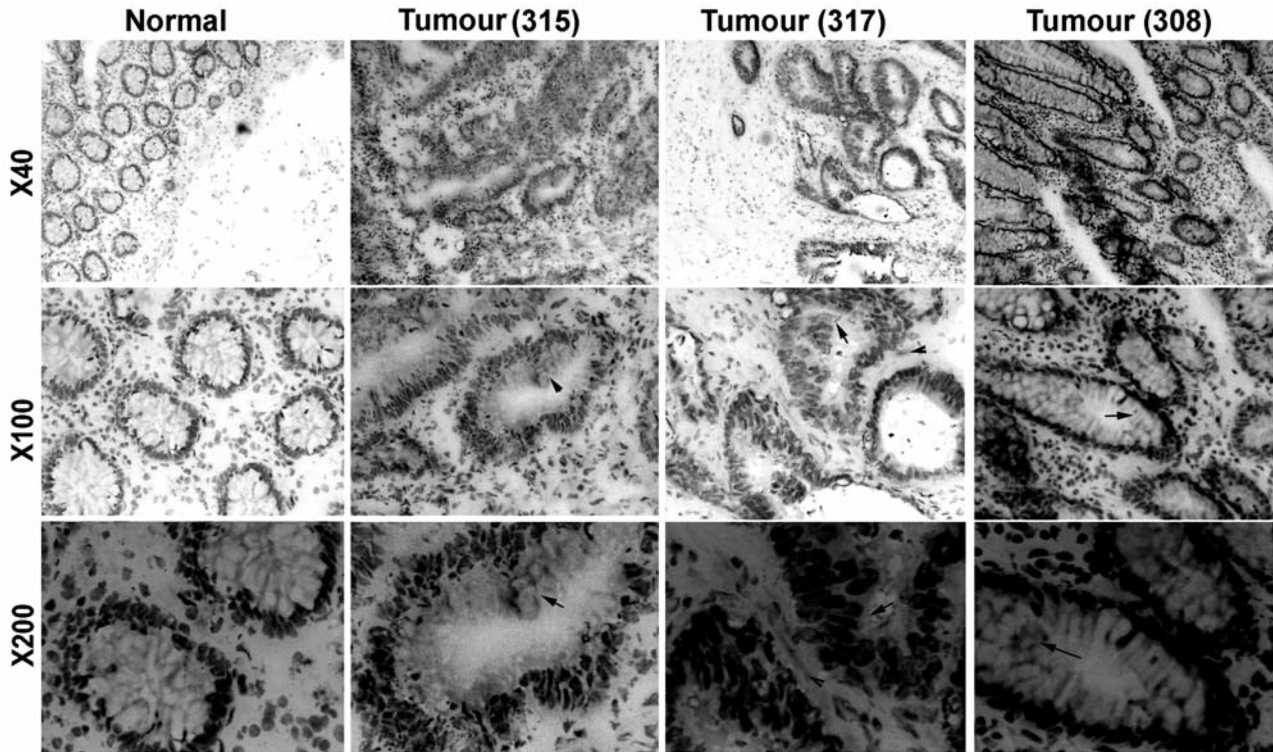

Figure 1. *Immunochemical staining of SIPA1 in colorectal tissues. Representative images of SIPA1 staining in colorectal tissues are shown, including normal background, tumours 315, 317 and 308 of Dukes A, B and C, respectively. Arrows indicate the staining of SIPA1 seen in the cytoplasm of cancer cells. Arrowheads point to the staining in the cytoplasm of stromal cells.*

named HT115 and Caco-2 respectively, using constructed ribozyme transgenes targeting human SIPA1. The knockdown of *SIPA1* was verified using RT-PCR (Figure 3). The influence of *SIPA1*-knockdown on cellular functions was then determined. The effect on cell growth was firstly examined and reduced cell growth was seen of both HT115 and Caco-2 cells after knockdown of *SIPA1* (p<0.05), compared to the controls (Figure 4E and 4F). Invasiveness is considered to be a vital capability for tumour cells to disseminate locally and develop a metastasis (16). We examined the influence of *SIPA1* on the invasive nature of these colorectal cancer cells. Interestingly, knock-down of *SIPA1* expression resulted in a dramatic increase of invasive ability of HT115 (p<0.01) and Caco-2 cells (Figure 4A and 4B). Compared to the effect on invasion, knockdown of *SIPA1* exhibited a relatively weak impact on cell matrix adhesion. The number of cells adherent to Matrigel over a culture period of 40 min was slightly increased in SIPA1 knowdown of both HT115 and Caco-2 cell lines. Figure 4C shows adhesion of HT115 cells. *SIPA1* knockdown resulted in increased migration of both HT115 and Caco-2 cell lines compared with the respective controls. The effect of *SIPA1* knockdown on migration of Caco-2 cells is shown in Figure 4D.

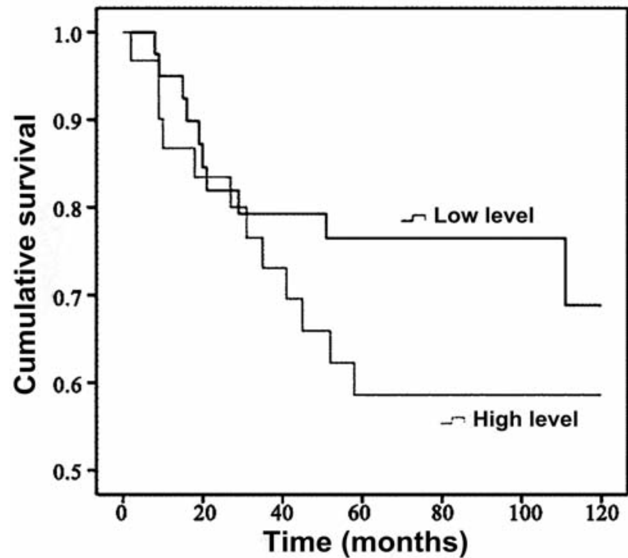

Figure 2. *Transcript levels of SIPA1 and overall survival. The average SIPA1 transcript level of Dukes' B group was used as a threshold.*

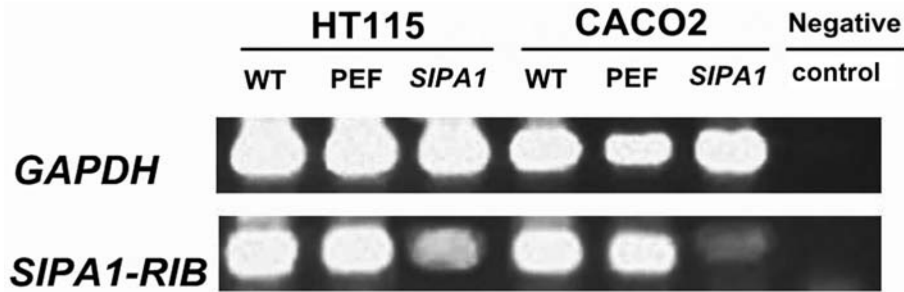

Figure 3. *Knockdown of SIPA1 in colorectal cancer cells was verified using RT-PCR. WT: Wild type; PEF: plasmid vector control.*

Figure 4. *Effect of SIPA1 knockdown SIPA1-RIB on functions of colorectal cancer cells. A: Knockdown of SIPA1 promoted invasiveness of HT115 cells. B: Influence on invasion of Caco-2 cells by SIPA1 knockdown. C: SIPA1 knockdown exhibited little effect on cell matrix adhesion. D: Impact on cell migration by SIPA1 knockdown was determined using a wounding assay. E: Knockdown of Sipa1 in HT115 cells resulted in reduced in vitro growth. F: SIPA1 knockdown inhibited in vitro growth of Caco-2 cells. Asterisk indicates p<0.01.*

Discussion

SIPA1 has been demonstrated to be a negative regulator for RAP GTPases. Members of the RAP family have been indicated in leukemia, as recently reviewed by Minato *et al*. (11). Knockdown of *Sipa1* in mice results in an aberrant activation of RAP, leading to the development of chronic myeloproliferative disorders and leukemia (17-19). In addition to leukemia (19), up-regulation of *Sipa1* has also been indicated in the disease progression of breast, prostatis and cervical cancer (9, 10, 13). In the current study, we examined the expression of *SIPA1* in colorectal cancer using quantitative real-time PCR and immunohistochemical staining. Elevated transcript levels of *SIPA1* were seen in the tumours compared to the background tissues. Except for this increased expression in colorectal tumours, no significant link was indentified between *SIPA1* expression and the disease features, including TNM staging, local recurrence, metastasis and survival. SNPs of *SIPA1* have been associated with aggressive disease behaviour in breast cancer (13). Whether SNPs of *SIPA1* also occur in colorectal cancer is yet to be investigated. Although not reaching statistical significant levels, *SIPA1* expression appeared to be lower in cancer cells with poorer differentiation and in tumours with lymphatic metastases. This is different from the finding in prostate cancer, in which increased expression of *SIPA1* has been associated with lymphatic metastasis (9). It suggests that diverse and dynamic roles are played by this molecule in colorectal cancer.

We transfected anti-human *SIPA1* hammerhead ribozymes into two colorectal tumor cell lines (HT115 and Caco-2), so as to deduce the role of *SIPA1* through *in vitro* cell function assays. A resulting increase was seen in the invasiveness of colorectal cancer cells following the knockdown of SIPA1. Similarly, but to a less degree, migration of the colorectal cancer cells was increased after *SIPA1* knockdown. It has been demonstrated that up-regulation of *SIPA1* in prostate cancer cells, results in reduced cell adhesion but increased invasion *via* its effect upon RAP signalling (9). In contrast, abnormal activation of RAP1 has also been associated with increased cell migration and invasiveness of prostate cancer cells (12). However, whether and how RAP1 is actually involved in the promotory effect of *SIPA1* knockdown, on the migration and invasion of colorectal cancer cells, warrants further investigations.

The present study has demonstrated that knockdown of *SIPA1* had an inhibitory effect on the *in vitro* growth of colorectal cancer cells. RAP1 has been indicated in the regulation of type 2 receptor of Transforming growth factor β, sensitizing pancreatic cancer cells to inhibition by TGF-β (20). However, the exact mechanisms and pathways involved in the effects of *SIPA1*-knockdown are yet to be investigated.

In conclusion, this study demonstrates that *SIPA1* expression is increased in human colorectal cancer. Knockdown of *SIPA1* expression enhances the capability of both HT115 and Caco-2 colorectal cancer cell lines to become invasive and migratory, but reduced their *in vitro* growth. It is concluded that *SIPA1* may play an active role during the disease progression of colorectal adenocarcinoma.

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References

1 Dukes CE and Bussey HJ: The spread of rectal cancer and its effect on prognosis. Br J Cancer *12*: 309-320, 1958
2 Chapuis PH, Dent OF, Fisher R, Newland RC, Pheils MT, Smyth E and Colquhoun K: A multivariate analysis of clinical and pathological variables in prognosis after rescetion of large bowel cancer. Br J Cancer *72*: 1424-1429, 1985
3 Hunter KW, Broman KW, Voyer TL, Lukes L, Cozma D, Debies MT, Rouse J and Welch DR: Predisposition to efficient mammary tumor metastatic progression is linked to the breast cancer metastasis suppressor gene Brms1. Cancer Res *61*: 8866-8872, 2001.
4 Gaudet MM, Hunter K, Pharoah P and Dunning AM: Genetic variation in *SIPA1* in relation to breast cancer risk and survival after breast cancer diagnosis. Int J Cancer *124(7)*: 1716-1720, 2009.
5 Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L, Sicotte H, Staats B, Acharya M, Crenshaw A, Eckert A and Puri V: SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Res *34*: D617-D621, 2006.
6 Crawford NP, Ziogas A, Peel DJ, Hess J, Anton-Culver H and Hunter KW: Germline polymorphisms in SIPA1 are associated with metastasis and other indicators of poor prognosis in breast cancer. Breast Cancer Res *8*: R16, 2006.
7 Page DL: Prognosis and breast cancer.Recognition of lethal and favorable prognostic types. Am J SurgPathol *15*: 334-349, 1991.
8 Platet N, Cathiard AM, Gleizes M and Garcia M: Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. Crit Rev Oncol Hematol *51*: 55-67, 2004.
9 Shimizu Y, Hamazaki Y, Hattori M, Doi K, Terada N, Kobayashi T, Toda Y, Yamasaki T, Inoue T, Kajita Y, Maeno A, Kamba T, Mikami Y, Kamoto T, Yamada T, Kanno T, Yoshikawa K, Ogawa O, Minato N and Nakamura E: *SPA-1* controls the invasion and metastasis of human prostate cancer. Cancer Sci *102*: 828-836, 2011.
10 Brooks R, Kizer N, Nguyen L, Jaishuen A, Wanat K, Nugent E, Grigsby P, Allsworth JE and Rader JS: Polymorphisms in *MMP9* and *SIPA1* are associated with increased risk of nodal metastases in early-stage cervical cancer. Gynecol Oncol *116*: 539-543, 2010.
11 Minato N and Hattori M: *Spa-1* (*Sipa1*) and RAP signaling in leukemia and cancer metastasis. Cancer Sci *100*: 17-23, 2009.
12 Bailey CL, Kelly P and Casey PJ: Activation of RAP1 promotes prostate cancer metastasis. Cancer Res *69*: 4962-4968, 2009.

13 Crawford NP, Ziogas A, Peel DJ, Hess J, Anton-Culver H and Hunter KW: Germline polymorphisms in *SIPA1* are associated with metastasis and other indicators of poor prognosis in breast cancer. Breast Cancer Res *8*: R16, 2006.
14 Zuker M: Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res *31*: 3406-3415, 2003.
15 Jiang WG, Grimshaw D, Lane J, Martin TA, Abounader R, Laterra J and Mansel RE: A hammerhead ribozyme suppresses expression of hepatocyte growth factor/scatter factor receptor c-MET and reduces migration and invasiveness of breast cancer cells. Clin Cancer Res *7*: 2555-2562, 2001.
16 Ye L, Bokobza S, Li J, Moazzam M, Chen J, Mansel RE and Jiang WG: Bone morphogenetic protein-10 (*BMP-10*) inhibit saggressiveness of breast cancer cells and correlates with poor prognosis in breast cancer, Cancer Science *101*: 2137-2144, 2010.
17 Ishida D, Kometani K, Yang H, Kakugawa K, Masuda K, Iwai K, Suzuki M, Itohara S, Nakahata T, Hiai H, Kawamoto H, Hattori M and Minato N: Myeloproliferative stem cell disorders by deregulated Rap1 activation in Spa-1-deficient mice. Cancer Cell *4*: 55-65, 2003.
18 Ishida D, Yang H, Masuda K, Uesugi K, Kawamoto H, Hattori M and Minato N: Antigen-driven T cell anergy and defective memory T-cell response *via* deregulated RAP1 activation in Spa-1-deficient mice. Proc Natl Acad Sci USA *100*: 10919-10924, 2003.
19 Kometani K, Aoki M, Kawamata S, Shinozuka Y, Era T, Taniwaki M, Hattori M and Minato N: Role of *SPA-1* in phenotypes of chronic myelogenous leukemia induced by BCR-ABL-expressing hematopoietic progenitors in a mouse model. Cancer Res *66*: 9967-9976, 2006.
20 Fralix KD, Zhao S, Venkatasubbarao K and Freeman JW: RAP1 reverses transcriptional repression of TGF-β type II receptor by a mechanism involving AP-1 in the human pancreatic cancer cell line, UK Pan-1. J Cell Physiol *194*: 88-99, 2003.

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