Chapter

# A Novel SASH1-IQGAP1-E-Cadherin Signal Cascade Mediates Breast Cancer Metastasis

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## Abstract

SAM and SH3 domain-containing protein 1 (SASH1) was previously described as a candidate tumor suppressor gene in breast cancer and colon cancer to mediate tumor metastasis and tumor growth. However, the underlying mechanism that SASH1 implements breast cancer metastasis in most solid cancers remains unexplored. In this study, SASH1 was identified to bind to IQ motif-containing GTPase activating protein 1 (IQGAP1). In breast cancer tissues, there was a correlation between the expressions of SASH1 and IQGAP1 ($P < 0.05$), and the expressions of SASH1 and IQGAP1 proteins were, respectively, correlated with the expression of E-cadherin ($P < 0.001$). In addition, the expressions of SASH1 and IQGAP1 proteins were correlated with tumor diameter and tumor grade (all $P < 0.05$) but without lymph node metastasis ($P > 0.05$). Therefore, it is suggested that SASH1 may form a new signaling cascade with IQGAP1 and E-cadherin to regulate breast cancer metastasis.

**Keywords:** breast neoplasms, gene expression regulation, cadherins, SAM and SH3 domain-containing protein 1 (SASH1), IQ motif-containing GTPase activating protein 1 (IQGAP1)

## 1. Introduction

Breast neoplasm is the most common cancer in women, which is originated from mammary epithelial tissue. The age of breast cancer is about 40–60 years old or before and after menopause. The morbidity of breast cancer is showed to be an upward trend year by year [1]. There are many factors that trigger breast cancer; however, the genetic factors only account for 10 and 90% of inducing factors of breast cancer remain to be investigated. SASH1 is a novel tumor suppressor gene, which is located in chromosome 6q24.3 [2] and is expressed in most of human tissues and cells except for lymphocytes and dendritic cells [3]. SASH1 was originally identified as a candidate tumor suppressor gene in breast cancer and colon cancer, regulating tumorigenesis of breast and other solid cancers and the adhesive and migratory behavior of cancer cells in tumor formation [4, 5]. Compared with that in normal breast epithelial tissues, SASH1 is downregulated in 74% of epithelial tissues of breast cancer-affected individuals [4, 6]. Some studies indicate that SASH1 downregulation is associated with tumor metastasis [3, 5]. Other studies

indicate downregulated SASH1 promotes metastasis of hepatoma carcinoma cells through Shh signal pathway [7].

IQGAP1 is a scaffolding protein with 189 kDa of molecule weight, which contains multiple protein-interacting domains, such as a calponin homology domain, a polyproline-binding domain, four calmodulin-binding motifs, and a Ras GAP-related domain [8, 9]. The binding players of IQGAP1 proteins are involved in actin, calmodulin, members of the Rho GTPase family (i.e., Rac1 and Cdc42), Rap1, E-cadherin, β-catenin, members of the mitogen-activated protein kinase (MAPK) pathway, and adenomatous polyposis coli [8, 10]. Various basic cellular activities such as cytoskeletal organization, cell-cell adhesion, cell migration, transcription, and signal transduction are mediated by the bindings of IQGAP1 to these proteins [11]. Cell-cell adhesion of epithelial cells is predominantly mediated by E-cadherin and the associated catenin complex [12], which includes α-catenin (102 kDa), β-catenin (92 kDa), and γ-catenin/plakoglobin (83 kDa). β-Catenin combines with E-cadherin, and α-catenin links this E-cadherin/β-catenin complex to the actin cytoskeleton, which is essential for E-cadherin to express its full adhesive function. Remodeling of this adhesive sequence leads to cell detachment or loosening of cell-cell contact, which promotes epithelial cells to move as clusters, and IQGAP1 is involved in the remodeling of the adhesive complexes of epithelial cells [11, 13–15]. Our previous studies suggest that SASH1 is associated with MAP2K2 to cross talk with ERK1/2-CREB cascade to trigger melanin synthesis in the formation of hyperpigmentation plaques of a kind of dyschromatosis [16]. Importantly, our previous studies also indicate that SASH1 not only bind to G alpha S protein (Gαs) but IQGAP1 to form a novel Gαs-SASH1-IQGAP1-E-cadherin cascade and mutated SASH1(s) which mediate E-cadherin expression through the Gαs-SASH1-IQGAP1-E-cadherin cascade to promote directional migration of melanocytes or melanoma cells [17]. So, it is speculated that this mechanism may also exist in breast cancer cells. Taken above, the associations between SASH1 and IQGAP1 in breast cancer cells and the expression of SASH1, IQGAP1, and E-cadherin were analyzed by immunohistochemistry analyses in 80 cases of the affected individuals of breast cancer. Furthermore, the expression relationship among SASH1, IQGAP1, and E-cadherin and the associations between clinical index of breast cancer patients and the expression of SASH1 and IQGAP1, respectively, were assessed to find out novel interference targets for early prevention of breast cancer metastasis.

## 2. Material and methods

### 2.1 Plasmid construction of HA-IQGAP1-pcDNA3.0 and pEGFP-C3-SASH1

The construction of pEGFP-C3-SASH1 recombined vectors was mainly referred to our previous description [17]. IQGAP1 cDNA was obtained from Han Jiahuai Lab, Xiamen University (Xiamen, Fujian, China), and cloned into pcDNA3.0-HA vector. PCR was performed with IQGAP1 cDNA as template using TransTaq® DNA Polymerase High Fidelity (TransGen Biotech, Ltd., Beijing, China) using the following cloning primers of IQGAP1: sense primer, 5'-TAGTCTAGAAT GTCCG CCGCAACGAG-3'(Xba I inserted) and antisense primer, 5′-CCGCTCGAGTTACTTCCCGTAGAACTTTTTG-3′ (Xho I inserted). The amplification conditions were as follows: 95°C 2 min, 95°C 30 s, 58°C 30 s, and 72°C 1 min for 30 cycles and 72°C 5 min and 4°C forever. The recombined vectors were identified by enzyme digestion of endonuclease and CDS of SASH1 and IQGAP1 genes.

### 2.2 Cell culture and transfection

Human breast cancer cell lines including SK-BR-3 cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). After several times of passage, cells were used and cultured in Dulbecco's Modified Eagle's medium (DMEM) (Gibco, Logan, UT), containing 10% BI fetal bovine serum (Bioind, Israel) and 1% penicillin-streptomycin solution at 37°C with 5% $CO_2$. SK-BR-3 cells were subcultured for three times and cultured to logarithmic growth phase for plasmid transfection. The HA-IQGAP1-pcDNA3.0 and pEGFP-C3-SASH1 were transfected into SK-BR-3 cells according to different combinations using PEI prepared by us. The transfected SK-BR-3 cells were divided into three groups, that is, two single-vector transfection groups and one double-vector transfection group. At 48 h after transfection, the transfected SK-BR-3 cells were lysed and collected for immunoprecipitation assays.

### 2.3 Immunoprecipitation and immunoblotting

Transfected SK-BR-3 cells were gently washed in PBS three times and then lysed for 25 min using IP-WB lysis buffer (Beyondtime Inc. Ltd., Jiangsu, China) with complete protease inhibitor cocktail per 10-cm dish for 20 min on ice. The cell lysates were transferred to 1.5 ml microcentrifuge tubes. The extracts were centrifuged for 15 min at 12,000 rpm at 4°C. The supernatants were immunoprecipitated using GFP mouse monoclonal antibody (T0005, Affinity Biosciences, Cincinnati, OH, USA) or HA mouse monoclonal antibody (mAb) (Abmart, Shanghai, China) as performed in our previous descriptions [17]. The immunoprecipitates were washed with PBS for three times and subjected to western blotting as previously described [16, 17]. Most of the western blots were mainly performed in our previous reports [17]. The associated HA-IQGAP1 or GFP-SASH1 was detected by western blot along with β-tubulin as loading control. The primary antibodies used in western blot were as follows: anti-GFP, anti-HA, and anti-β-tubulin (10B1) mouse mAb (EarthOx Life Science, Millbrae, CA, USA or Shanghai Genomics, Shanghai, China).

### 2.4 Clinical cases

All breast cancer patients who underwent surgery were followed by treatment in accordance with the National Comprehensive Cancer Network clinical practice guidelines. Fresh primary breast cancer tissues and some of the corresponding adjacent tissues were collected from 80 breast ductal carcinoma patients undergoing resection from May 2015 to June 2016 at the Chongqing Cancer Hospital. Histological diagnosis and tumor-node-metastasis staging of cancer were determined in accordance with the American Joint Committee on Cancer manual criteria for breast cancer. Written informed consent regarding tissue and data used for scientific purposes was obtained from all participating patients. The study was approved by the Research Ethics Committees of the affiliated Hospitals of Guizhou Medical University and Chongqing Cancer Hospital. All of the breast cancer cases were diagnosed by pathological examinations (HE staining and immunohistochemistry analyses). In the clinical cases of breast cancer, 26 cases are with lymph node metastasis, 51 cases are without lymph node metastasis, and 3 cases could not acquire the information of lymph node metastasis. Breast tumor diameters of 16 cases were <1 cm, those of 41 cases were 1.1–2 cm, those of 18 cases were 2.1–3 cm, and those of 5 cases were >3 cm. According to WHO histological classification of breast tumors (2003), 80 cases of breast invasive ductal carcinoma were graded histologically in terms of duct formation, nuclear pleomorphism, and mitosis. Among

the 80 cases of breast invasive ductal carcinoma, 65 cases were graded into 3 grades: 8 cases belonged to grade I, 47 cases to grade II, and 10 cases to grade III.

### 2.5 Immunohistochemical analyses of SASH1, IQGAP1, and E-cadherin

The breast cancer tissues obtained from surgical operation were fixed at 4°C in 10% formaldehyde solution for 24 h. The excess fat and other tissues of breast cancer tissues were removed and embedded with paraffin and made into 5 millimeter (mm) tissue sections. The tissue sections (5 mm) were baked at 56°C and dehydrated and subjected to peroxidase blocking. Tissues of human breast cancer and corresponding adjacent tissues were immunohistochemically stained with SASH1 rabbit polyclonal antibody (pAb) (A302-265A-1, Bethyl Laboratories, Inc., Texas, USA, or Novus Biologicals, USA), IQGAP1 rabbit polyclonal antibody (Bethyl Laboratories, Inc., Texas, USA), and E-Cadherin (24E10) Rabbit mAb (#3195, Cell Signaling Technology). Primary antibodies were added and incubated at 37°C and then for overnight at 4°C. After washing three times for 10 min each with TBS, the sections were incubated with horseradish peroxidase-conjugated anti-rabbit and anti-mouse universal secondary antibodies for 30 min at 37°C. Subsequently, the sections were counterstained with hematoxylin mounted, observed, and photographed under the positive position microscope BX51 at a 100× magnification or a 400× magnification. Finally, the stained slides were observed under a microscope, and images were acquired [17]. The experimental protocols were mainly referred to our previous description [17].

According to the staining intensity of tumor cells, the three proteins, SASH1, IQGAP1, and E-cadherin, were scored and divided into four grades: 0 score (−), 1 score (+), 2 score (++), and 3 score (+++). The three proteins were also scored according to positive cells' percentage of the three proteins and divided into six grades: 0 score (<1%), 1 score (1–20%), 2 score (21–40%), 3 score (41–60%), 4 score (61–80%), 5 score (81–100%). Based on the staining intensity of SASH1, IQGAP1, and E-cadherin, the staining intensity and positive cells' percentage of three proteins were calculated as in our previous description [16]. Total scores of each visual field were determined by the formula: staining intensity scores of positive cells × scores of positive cells' percentage = total scores of each view fields.

### 2.6 Statistical analyses

All of experimental results were repeated for three times and statistically analyzed using SPSS 16.0 statistical software. Chi square test was performed to analyze the IHC results of breast cancer tissues and the relationship between expression of SASH1 and IQGAP1 and clinical indicators. Rank-sum test was used to assess the grading relationship between the SASH1 and E-cadherin and IQGAP1 and E-cadherin, respectively. Spearman correlation coefficient method was used to assess the correlation between expressed scores of SASH1 and E-cadherin and IQGAP1 and E-cadherin, respectively. The data are indicated as mean ± standard error of the mean (SEM), and the difference was statistically significant with $P < 0.05$. Cartograms were plotted with GraphPad Prism 5.

## 3. Results

### 3.1 SASH1 is associated with IQGAP1

To identify the associations between SASH1 and IQGAP1, HA-IQGAP1-pcDNA3.0 and pEGFP-C3-SASH1 were constructed and were singly or combinedly transfected

into SK-BR-3 cells and immunoprecipitation; western blot (IP-WB) was performed to identify the associations between exogenous SASH1 and exogenous IQGAP1. HA-IQGAP1 and GFP-SASH1 were singly or in pair transfected into SK-BR-3 cells at 48 h posttransfection, the transfected cells were lysed, and HA-IQGAP1 was immunoprecipitated, and the associated GFP-SASH1 was detected by GFP antibody. The associated HA-IQGAP1 and GFP-SASH1 in the immunoprecipitates and cell lysates (input) were confirmed by western blot. Meanwhile, GFP-SASH1 and HA-IQGAP1 were also either single or in pair transfected into SK-BR-3 cells and after 48 h of transfection, the transfected cells were lysed and were GFP-SASH1 was immunoprecipitated and the associated HA-IQGAP1 was detected by HA antibody. The associated GFP-SASH1 and HA-IQGAP1 in the immunoprecipitates and cell lysates (input) were identified by western blot. Finally, our IP-WB analyses confirmed that exogenous SASH1 was associated with exogenous IQGAP1 (**Figure 1**).

### 3.2 There was a positive correlation between SASH1 and IQGAP1 expression in breast cancer tissues

IHC analyses confirmed that the positive staining of SASH1 and IQGAP1 protein was light brown in breast cancer tissues, the cell nucleus was purple, and the distribution of SASH1 and IQGAP1 was located in the same sites of breast cancer tissues. SASH1 and IQGAP1 show the same or similar expression tendency in breast cancer tissues, i.e., low level of SASH1 expression is followed by low level of IQGAP1 expression and high expression of SASH1 is accompanied by high expression of IQGAP1 (**Figure 2A**). A total of 80 breast cancer tissues were divided into four groups according to the median value of SASH1 and IQGAP1 protein expression scores: SASH1 scores <1.23 were considered as low expression, SASH1 scores ≥1.23 were considered as high expression, IQGAP1 scores <0.78 were maintained as low expression, and SASH1 scores ≥0.78 were maintained as high expression. Statistical analyses indicated that in the 80 cases of breast cancer tissues, cases with low SASH1 expression accounted for 56.3% (45/80) and the cases with low IQGAP1 expression were more than those of high IQGAP1

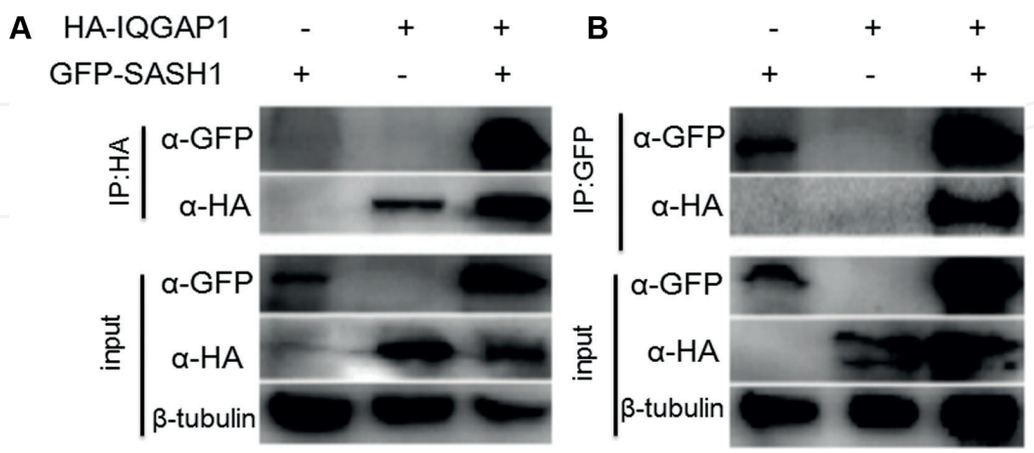


**Figure 1.**
*SASH1 is associated with IQGAP1. (A) GFP-SASH1 and HA-IQGAP1 were singly or in pair transfected into SK-BR-3 cells, and at 36 h after transfection, the transfected cells were lysed and collected for IP-WB analyses. HA-IQGAP1 was immunoprecipitated, and the associated GFP-SASH1 was detected by western blot using GFP antibody. GFP-SASH1 and HA-IQGAP1 in the cell lysates (input) were detected by western blot along with β-tubulin with loading control. (B) HA-IQGAP1 and GFP-SASH1 were singly or in pair transfected into SK-BR-3 cells, and at 36 h after transfection, the transfected cells were lysed and collected for IP-WB analyses. GFP-SASH1 was immunoprecipitated, and the associated GFP-SASH1 was detected by western blot using GFP antibody. HA-IQGAP1 and GFP-SASH1 in the cell lysates (input) were analyzed by western blot along with β-tubulin with loading control.*

expression (>65%, P = 0.015) (**Figure 2B**). And statistical analyses also suggested that in the 80 cases of breast cancer tissues, cases with low IQGAP1 expression accounted for 58.8% (47/80) and the cases with low IQGAP1 expression were more than those of high IQGAP1 expression (>60%, P = 0.011) (**Figure 2B**). Meanwhile, the IHC detection results of SASH1 and IQGAP1 were scored and analyzed by Spearman correlation analyses, and the scores of SASH1 and IQGAP1 were plotted by GraphPad Prism 5 software. In 80 cases of breast cancer tissues, except for 5 cases, SASH1 scores and IQGAP1 scores in most of cases closely

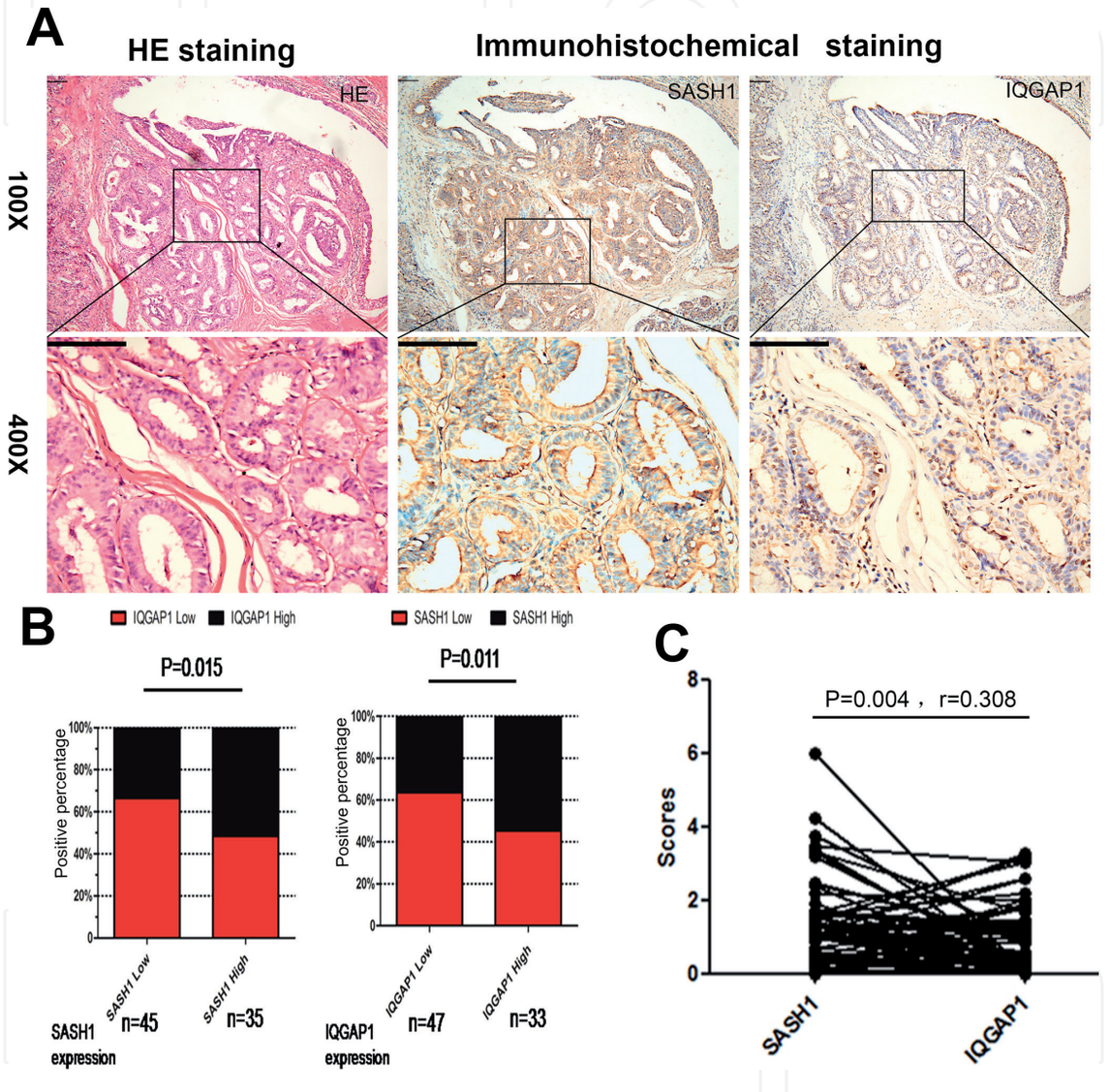


**Figure 2.**
*SASH1 expression in 80 cases of breast cancer tissues which is positively correlated with IQGAP1. (A) The expressions of SASH1 and IQGAP1 in 80 cases of breast cancer tissues were detected by immunohistochemical staining method. The cell nucleus was dyed purple and SASH1 and IQGAP1 were dyed pale brown. The left panels were HE staining, and the middle panels and the right panels were IHC staining of SASH1 and IQGAP1. The figures in upper panels were 100× magnification, and one region in the 100× magnification figures was amplified for 400× and framed in black and showed in the bottom panels. (B) The expressions of SASH1 and IQGAP1 were scored, and the score results of SASH1 and IQGAP1 were plotted with GraphPad Prism 5 and analyzed by $\chi^2$ test. The analysis results of SASH1 and IQGAP1 expressions in the left panel indicated that when SASH1 expression was low, the positive percentage of low expressed IQGAP1 was much more than that of high expressed IQGAP1. The expression of SASH1 showed significantly positive correlation with that of IQGAP1 (P = 0.015). And the statistical analyses also suggested that when IQGAP1 expression was low, the positive percentage of low expressed SASH1 was much more than that of high expressed SASH1. The expression of IQGAP1 demonstrated significantly positive correlation with that of SASH1 (P = 0.011). (C) The expressions of SASH1 and IQGAP1 were scored, and the score results of SASH1 and IQGAP1 were plotted with GraphPad Prism 5 and analyzed by Spearman correlation coefficient analyses. Spearman correlation coefficient analyses indicated that except for two score values of SASH1, expression of SASH1 and IQGAP1 showed good similar or same tendency of changes (P = 0.004).*

intersected, which indicated that the SASH1 expression and IQGAP1 expression showed significantly positive correlation (r = 0.308, P = 0.004) (**Figure 2C**).

### 3.3 The expression of SASH1 and IQGAP1 protein in breast cancer was significantly correlated with tumor size and tumor grade

It has been known that the expression of SASH1 and IQGAP1 is associated with tumor metastasis. So, in this study, we further identify the relationship of expression of SASH1 and IQGAP1 with clinical data of breast cancer-affected individuals. Our analyses (**Table 1**) indicated that in 77 cases of breast cancer with lymph node dissection, the low expression rate of SASH1 protein in lymph node metastasis positive group was slightly higher than that in lymph node metastasis negative group. The low

| Clinical parameters | Total | SASH1 | | IQGAP1 | | P |
|---|---|---|---|---|---|---|
| | | Low | High | Low | High | |
| Lymph node metastasis[a] | | | | | | >0.05 |
| Positive | 26 | 17 | 9 | 15 | 11 | |
| Negative | 51 | 28 | 23 | 30 | 21 | |
| Tumor diameter/cm[b] | | | | | | <0.05 |
| ≤1 | 16 | 8 | 8 | 10 | 6 | |
| 1.1–2 | 41 | 24 | 17 | 23 | 18 | |
| 2.1–3 | 18 | 9 | 9 | 10 | 8 | |
| >3 | 5 | 4 | 1 | 4 | 1 | |
| Histological grade[c] | | | | | | <0.01 |
| I | 8 | 4 | 4 | 6 | 2 | |
| II | 47 | 26 | 21 | 24 | 23 | |
| III | 10 | 7 | 3 | 7 | 3 | |

[a]*N = 77.*
[b]*N = 80.*
[c]*N = 65.*

**Table 1.**
*Association of SASH1 and IQGAP1 expressions with the clinical parameters of breast cancer patients (n).*

| E-cadherin | SASH1 | | | | | IQGAP1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | − | + | ++ | ++ | Total[a] | − | + | ++ | +++ | Total[b] |
| − | 4 | 6 | 6 | 1 | 17 | 8 | 8 | 1 | 0 | 17 |
| + | 6 | 20 | 2 | 0 | 28 | 12 | 12 | 4 | 0 | 28 |
| ++ | 5 | 25 | 3 | 0 | 33 | 12 | 19 | 2 | 0 | 33 |
| +++ | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 2 |
| Total | 16 | 51 | 12 | 1 | 80 | 33 | 40 | 7 | 0 | 80 |

[a]*r = 0.461, P < 0.001.*
[b]*r = 0.454, P < 0.001; by rank-sum test.*

**Table 2.**
*Correlation of SASH1 and IQGAP1 expressions with E-cadherin expression rankin breast cancer tissues (n, N = 80).*

expression rate of IQGAP1 protein was slightly lower than that of the negative lymph node metastasis group, but the difference was not statistically significant (65.4% vs. 54.9%, 57.7% vs. 58.8%, all P value > 0.05). In 80 cases of breast cancer, the low expression rate of SASH1 protein was 50.0, 58.5, 50.0, and 80.0%, respectively, in patients with tumor diameter <1.0 cm, 1.1–2.0 cm, 2.1–3.0 cm, and >3.0 cm. The low expression rates of IQGAP1 protein were 62.5, 56.1, 55.6, and 80.0%, respectively. There were significant differences between the two groups ($P < 0.05$). In 65 cases of breast cancer with histological grading data, the low expression rates of SASH1 protein in histological grading I, II, and III were 50.0, 55.3, and 70.0%, respectively. The low expression rates of IQGAP1 protein were 75.0, 51.1, and 70.0%, respectively. There were significant differences between groups ($P < 0.01$).

### 3.4 The expression of SASH1 and IQGAP1 is positively related with E-cadherin, respectively, in breast cancer tissues

SASH1 and IQGAP1 have been identified to be involved in tumor metastasis. And immunohistochemistry (IHC) analyses were performed to detect the expression of E-cadherin in breast cancer tissues and the relevance of E-cadherin with SASH1 and IQGAP1, respectively. IHC analyses indicated that E-cadherin was mainly located in the cytoplasma membrane of breast cancer tissues. According to the positive intensity of E-cadherin staining, E-cadherin protein expression in breast cancer tissues was graded to four grades, and meanwhile the positive intensity of SASH1 protein staining was also graded to four grades. Statistical analyses suggested that expression of SASH1 protein was significantly positive related to that of E-cadherin ($r = 0.461$, $P < 0.001$ (**Table 2** and **Figure 3**)).

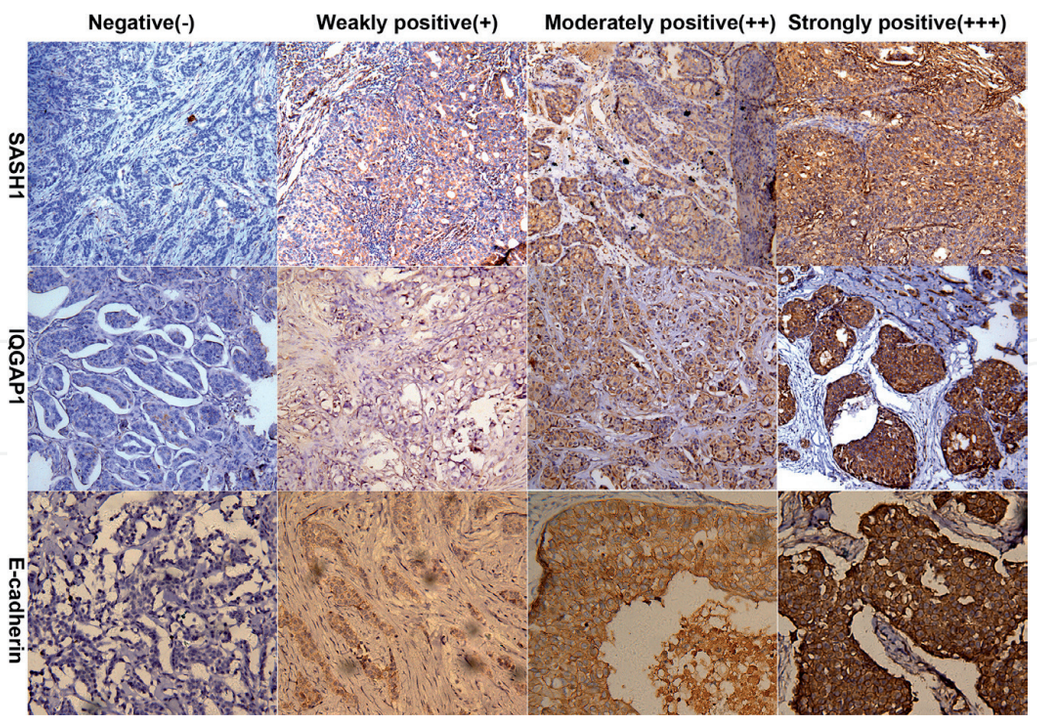


**Figure 3.**
*SASH1, IQGAP1, and E-cadherin proteins showed consistent changes in the breast cancer tissues. The cell nucleus was dyed purple and SASH1, IQGAP1, and E-cadherin were dyed pale brown, and the magnification is 200×. According to staining intensity of tumor cells and the numbers of positive cells, the immunohistochemical results of SASH1, IQGAP1, and E-cadherin proteins were divided into four grades: negative (−), weakly positive (+), moderately positive (++), and strongly positive (+++). The expression of SASH1 was positively correlated with that of E-cadherin and the expression of IQGAP1 show positive correlation with that of E-cadherin. The cell nucleus was dyed purple, and SASH1, IQGAP1, and, E-cadherin were dyed pale brown, and the magnification is 200×.*

SASH1 and E-cadherin staining intensity was moderately positive staining intensity, respectively, which was defined low expression. And further statistical analyses suggested SASH1 and E-cadherin were downregulated in 77 cases (77/80, 96.25%) of breast cancer tissues. All of these indicated that the low expression of SASH1 and the low expression of E-cadherin protein in breast cancer tissue are in good agreement.

According to the staining intensity of IQGAP1 protein and E-cadherin protein in 80 cases of breast cancer, the staining intensity of E-cadherin and IQGAP1 was divided into four grades. Statistical analyses demonstrated that expression of IQGAP1 protein was significantly positive related to that of E-cadherin ($r = 0.454$, $P < 0.001$ (**Table 2** and **Figure 3**)). Staining intensity of IQGAP1 and E-cadherin was moderately positive staining intensity, respectively, which was defined low expression. And further statistical analyses suggested IQGAP1 and E-cadherin were both downregulated in 78 cases (78/80, 97.5%) of breast cancer tissues. All of these indicated that the low expression of IQGAP1 and the low expression of E-cadherin showed better consistency in breast cancer tissue.

## 4. Conclusion

Clinical research indicates that occurrence of breast cancer is associated with many factors including genetic factors, environment, and lifestyle. SASH1, a tumor suppressor gene, is downregulated in most of neoplasms. Decrease or deletion of SASH1 expression is closely related to tumor metastasis [4, 5, 18]. It has been reported that the expression of SASH1 protein in osteosarcoma tissues with lung metastasis is significantly lower than that in osteosarcoma tissues without lung metastasis [19]. Upregulated SASH1 can significantly suppress the migration of cervical carcinoma Hela cells, and, in contrast, knockdown of SASH1 significantly results in reduced adhesion ability of human colon cancer SW480 cells and mouse rectal cancer CMT-93 cells and enhanced migration ability of these tumor cells [3]. Downregulation of SASH1 protein expression in thyroid tumor cells may play an important role in thyroid tumor metastasis [20]. SASH1 mRNA is downregulated in primary liver cancer and thyroid cancer [5]. Compared with corresponding normal tissues, SASH1 protein is downregulated in 37 cases among 50 cases of breast cancer tissues and SASH1 expression loss is associated with breast cancer metastasis [4]. All of these studies suggest that expression loss of SASH1 medicates tumor metastasis. In this study, our IHC analyses identified that in 80 cases of breast cancer tissues, low expression of SASH1 protein in 45 cases (45/80 56.3%) was found, which indicated that SASH1 was downregulated in breast cancer.

IQGAP1 proteins are members of the evolutionarily conserved scaffolding protein family and are more widely expressed than other members of the family [21, 22]. IQGAP1 interacts with specific proteins such as actin, calmodulin, Rho GTPase family members, E-cadherin, and β-catenin. The interactions of IQGAP1 with those specific proteins medicate multiple cell activities such as cell scaffold, intercellular adhesion, metastasis, invasion, transcription, and cell signal transduction. For example, the binding of IQGAP1 to β-catenin to form E-cadherin/β-catenin complex inhibits intercellular adhesion of epithelial cells and promotes β-catenin-mediated transcriptional activation [9]. IQGAP1 protein ,which mediates E-cadherin-mediated-intercellular adhesion, is the key molecule in cell polarization and directed migration [23]. IQGAP1 expression is showed to be of prognostic significance in advanced colorectal carcinoma, and a shorter overall survival of colorectal carcinoma patients can be predicted by diffuse

expression pattern of IQGAP1 [11]. In this study, IHC analyses indicated, in 80 cases of breast cancer tissues, IQGAP1 protein level was significantly low in 47 cases accounting for 58.8%, which suggested that IQGAP1 was downregulated in breast cancer.

Multiple endocrine neoplasia type 1 (MEN1) is a dominantly inherited tumor syndrome that results from the mutation of the MEN1 gene that encodes protein menin. MEN1 is revealed to bind to IQGAP1 and increases E-cadherin/β-catenin interaction with IQGAP1 and a novel menin-IQGAP1 pathway that controls cell migration and cell-cell adhesion found in endocrine cells [24]. Activated Rac1 and Cdc42 can bind to IQGAP1, and the bindings of IQGAP1 and Rac1 as well as Cdc42 promote cell mobility and polarization [25, 26]. IQGAP1 is both a downstream effector and an upstream activator of Cdc42, where active Cdc42 antagonizes IQGAP1 dissociation of the cell-cell contacts [27, 28]. Cdc42 inhibits IQGAP1's role in polarized secretion in β-cells or perhaps migration [29]. In this study, IP-WB analyses indicated the protein-protein interactions between SASH1 and IQGAP1. It has been reported that SASH1 expression suppresses cell proliferation and interacts with cytoskeletal proteins, which promotes cell matrix adhesion [3, 4]. Meanwhile, other studies have identified that SASH1 is associated with scaffold proteins and foster tumor migration [3]. Hence, we speculate that the bindings of SASH1 and IQGAP1 co-mediate breast cancer metastasis.

Recurrence or metastasis of breast cancer is the leading cause of breast cancer-related death. It has been identified that epithelial-mesenchymal transition (EMT) plays a pivotal role in tumor metastasis through generation and survival of induced circulating tumor cells [30]. One of the EMT functions is to downregulate and relocate the epithelial cell adhesion protein including the leading actor, E-cadherin [31]. The decreased expression of E-cadherin in breast cancer was associated with high pathological grade, tumor volume enlargement, lymph node metastasis, and distant metastasis and with disease rehabilitation and overall survival time, which indicates that reduced expression or function loss of E-cadherin promotes breast cancer invasion and migration [32]. A dynamic equilibrium of E-cadherin between the E-cadherin-β-catenin-α-catenin complex and the E-cadherin-β-catenin-IQGAP1 complex at sites of cell-cell contact is proposed. The ratio between these two complexes could determine the strength of adhesion [33]. Our previous study found that SASH1 mutations enhanced mutated SASH1 expression, however induced downregulation of E-cadherin in epithelial cells of skin [17]. So it is speculated that there is a connection between SASH1 and E-cadherin. In this study, SASH1 protein level is positively correlated with E-cadherin, and IQGAP1 protein level is also positively correlated with E-cadherin, which also identifies the connection between SASH1 and E-cadherin.

Taken above, we speculate that SASH1 may mediate breast cancer metastasis through a novel SASH1-IQGAP1-E-cadherin signal cascade. When SASH1 and IQGAP1 protein levels in breast cancer tissues and breast cancer cells were low, the protein levels of E-cadherin are also reduced, which causes the reduced cell adhesion ability, the tumor cell ability which is easy to fall off, the enhanced invasion, and the tumor cell metastasis ability to distance. The novel findings about SASH1 will become a novel target to treat breast cancer, which will be conducive to the precision and diversification of breast cancer treatment, effectively improving the prognosis of patients. In this study, we find that low protein levels of SASH1 and IQGAP1 are related to tumor size, and the reduced protein levels of SASH1 and IQGAP1 are associated with tumor grading, which provides a new reference for the rapid diagnosis of tumor grading and tumor size. And our findings on SASH1

and IQGAP1 provide a new and more intuitive basis for determining the operation plan and resection range, judging the curative effect of operation and early detection of tumor metastasis and recurrence. However, in this study we find that low expression levels of SASH1 and IQGAP1 are significantly not related to lymph node metastasis, which presumably can be related to a small sample size of breast cancer tissues. The relationship between SASH1 and IQGAP1 with lymph node metastasis needs to be further investigated.

## Acknowledgements

We thank Central Laboratory at Yongchuan Hospital, Chongqing Medical University and Clinical Research Center, and the Affiliated Hospital, Guizhou Medical University for housing experiments. We thank Zhou Xin and Jiang Xiaoli of the Breast Disease Diagnosis and Treatment Center of Chongqing Cancer Hospital for their active help and cooperation in sample collection. This work was supported partly by the High Level Talent Introduction Project of Affiliated Hospital of Guizhou Medical University (grant numbers: 2018I-1 and I-2017-19) and Guizhou Provincial Science and Technology Department Project (grant number: Qian Ke He LH [2017] 7193). This work was supported by the Yongchuan Hospital Project, Chongqing Medical University (YJYJ201347), Chongqing Education Commission Project (KJ1400201), and Guizhou's Introduction Project of Million Talents.

## Conflict of interest

No conflict between the authors.

## Notes/thanks/other declarations

Notes: The chapter text was mainly referred to our article entitled as "SASH1-IQGAP1-E-cadherin signal cascade may regulate breast cancer metastasis" (Tumor. 2017;37(6):633–641) which we published in the Chinese Journal Tumor in June 2017. In this chapter, we rewrite the chapter text according to the suggestions of reviewers.

The figures and tables of this chapter were taken or reedited from the figures and tables of our published article entitled "SASH1-IQGAP1-E-cadherin signal cascade may regulate breast cancer."

## Declarations

## Acronyms and abbreviations

| | |
|---|---|
| DMEM | Dulbecco's Modified Eagle's medium |
| EMT | epithelial-mesenchymal transition |
| Gαs | guanine nucleotide-binding protein subunit-alpha isoforms short |
| HE staining | hematoxylin and eosin staining |
| IHC | immunohistochemical |
| IQGAP1 | IQ motif-containing GTPase activating protein 1 |
| IP-WB | immunoprecipitation-western blot |
| PEI | polyethylenimine |
| pAb | polyclonal antibody |
| SASH1 | SAM and SH3 domain-containing 1 |
| SEMs | standard error of the means |



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