Overexpression of the Transmembrane Protein IQGAP3 Is Associated with Poor Survival of Patients with Gastric Cancer

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Keywords

IQGAP3 · Mucin phenotype · Spheroid · Cancer stem cell · Gastric cancer

Abstract

Objective: Spheroid colony formation is a useful method of cancer stem cell (CSC) characterization. We previously showed that the IQ motif containing the GTPase-activating protein 3 gene (*IQGAP3*) is upregulated in spheroid body-forming gastric cancer (GC) cells compared with parental cells. We investigated *IQGAP3* expression in GC. **Methods:** *IQGAP3* protein expression was analyzed by immunohistochemistry in 165 GC cases. RNA interference was used to inhibit *IQGAP3* expression in GC cell lines. **Results:** In non-neoplastic gastric mucosa, weak staining of *IQGAP3* was observed in the foveolar epithelium, while GC tissue showed stronger, more extensive staining. Of the 165 GC cases, 34 (21%) were positive for *IQGAP3* expression. GC cases positive for *IQGAP3* were found more frequently in stage II/III/IV cases than in stage I cases. Univariate and multivariate analyses demonstrated that *IQGAP3* expression is an independent prognostic classifier of GC patients. Both the number and size

of the spheres formed by MKN-74 cells were significantly reduced by knockdown of *IQGAP3*. The phosphorylation of Akt and Erk1/2 was inhibited by knockdown of *IQGAP3*. **Conclusion:** These results suggest that *IQGAP3* plays an important role in gastric CSCs. The location of *IQGAP3* on the cell membrane makes it a potential therapeutic target for GC.

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Introduction

Gastric cancer (GC) is one of the most common human cancers. Multiple genetic and epigenetic alterations are associated with GC, so enhanced knowledge of the gene expression changes that occur during gastric carcinogenesis may facilitate improvements in disease diagnosis, treatment, and prevention [1]. Genes encoding transmembrane/secretory proteins that are expressed specifically in cancers are ideal diagnostic biomarkers. Moreover, if the products of these genes function in the neoplastic process, they may also be suitable therapeutic targets [2].

During the past decade, cancer has been recognized as a stem cell disease [3], and cancer stem cells (CSCs) have

been described in numerous solid tumors. Gastric CSCs are characterized by the expression of specific cell surface markers including CD44, CD133, and aldehyde dehydrogenase 1 (ALDH1) [4]. However, gastric CSCs have not been completely characterized.

Spheroid colony formation is a useful method with which to characterize gastric CSCs [5]. Previously, we reported on the upregulation of genes in spheroid body-forming GC cell lines compared with parental cells [6]. We showed that KIFC1 and KIF11 protein expression is upregulated in 37 and 72%, respectively, of GC cases, and that both the number and size of spheres from GC cell lines are significantly reduced in *KIFC1* and *KIF11* small interfering (si)RNA-transfected cells compared with negative control siRNA-transfected cells [6, 7]. However, the expression of many genes remains unconfirmed, and their role in GC is unclear.

In the present study, we focused on the IQ motif containing the GTPase-activating protein 3 gene (*IQGAP3*), because we previously observed it to be upregulated in spheroid body-forming cells compared with parental cells [6]. Moreover, because *IQGAP3* is a transmembrane protein, it has the potential to be a therapeutic target [8]. The *IQGAP* family comprises 3 members, *IQGAP1*, *IQGAP2*, and *IQGAP3* [9], which all contain 4 IQ motifs and a Ras-GAP-related domain [10]. *IQGAP1* has been suggested to function in cell migration and regulation of the cytoskeleton [11], and it may play a role in cancer progression [12]. In contrast, *IQGAP2* seems to act as a tumor suppressor [13], while *IQGAP3* is thought to be involved in the proliferation of epithelial cells [14]. However, the role of *IQGAP3* in GC pathogenesis remains unclear.

Herein, we analyzed the expression and distribution of *IQGAP3* protein in human GC using immunohistochemistry, and examined the relationship between *IQGAP3* expression and clinicopathologic characteristics. Furthermore, because gastric and intestinal mucin phenotypes of GC have distinct clinical characteristics and exhibit specific genetic and epigenetic changes [1], associations between *IQGAP3* expression and gastric/intestinal mucin phenotypes were investigated. We also analyzed the effect of inhibiting *IQGAP3* expression by RNA interference (RNAi) on spheroid formation by GC cells.

Materials and Methods

Tissue Samples

In a retrospective study, samples from 175 primary tumors were collected from patients diagnosed with GC who underwent surgery between 2003 and 2008 at Hiroshima University Hospital

(Hiroshima, Japan). This study was approved by the Ethics Committee for Human Genome Research of Hiroshima University (Hiroshima, Japan). All patients underwent curative resection. Only patients without preoperative radiotherapy or chemotherapy and without clinical evidence of distant metastasis were enrolled in the study. Operative mortality was defined as death within 30 days of leaving hospital, and these patients were removed from the analysis. Postoperative follow-up was scheduled every 1, 2, or 3 months during the first 2 years after surgery and every 6 months thereafter unless more frequent follow-up was deemed necessary. Chest X-ray, chest computed tomography scan, and serum chemistries were performed at every follow-up visit. Patients were followed by their physicians until their death or the date of the last documented contact.

For quantitative reverse transcription (qRT)-PCR, 10 GC samples were used. Samples were frozen immediately in liquid nitrogen and stored at -80°C until use. For immunohistochemical analysis, archival formalin-fixed, paraffin-embedded tissues from 165 patients who had undergone surgical excision for GC were used. One or two representative tumor blocks, including the tumor center, invading front, and tumor-associated nonneoplastic mucosa, from each patient were examined using immunohistochemistry. In cases of large, late-stage tumors, 2 different sections were examined to include representative areas of the tumor center as well as of the lateral and deep tumor invasive fronts. Tumor staging was determined according to the tumor-node-metastasis (TNM) classification system [15].

qRT-PCR Analysis

Total RNA was extracted from GC samples using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and 1 μg of total RNA was converted to cDNA using the First Strand cDNA Synthesis Kit (Amersham Biosciences, Piscataway, NJ, USA). Quantitation of *IQGAP3* mRNA levels was performed by real-time fluorescence detection as described previously [16]. PCR was conducted using the SYBR Green PCR Core Reagents Kit (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Real-time detection of the emission intensity of SYBR Green bound to double-stranded DNA was performed with the ABI PRISM 7700 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.). *ACTB*-specific PCR products were amplified from the same RNA samples and served as an internal control.

Immunohistochemistry

Immunohistochemical analysis was performed with a Dako Envision+ Mouse/Rabbit Peroxidase Detection System (Dako Cytomation, Carpinteria, CA, USA). Antigen retrieval was performed by microwave heating in citrate buffer (pH 6.0) for 30 min. Peroxidase activity was blocked with 3% H_2O_2 -methanol for 10 min, and sections were incubated with normal goat serum (Dako Cytomation) for 20 min to block nonspecific antibody binding sites. Sections were incubated with a rabbit polyclonal anti-*IQGAP3* antibody (1:50, kindly provided by Prof. Sachiko Tsukita, Osaka University, Osaka, Japan) for 1 h at room temperature, followed by incubation with Envision+ anti-rabbit peroxidase for 1 h. For color reactions, sections were incubated with the DAB Substrate-Chromogen Solution (Dako Cytomation) for 10 min. Sections were counterstained with 0.1% hematoxylin. Reactions lacking a primary antibody were used as negative controls.

Fig. 1. Expression of IQGAP3 in gastric cancer (GC). **a** qRT-PCR analysis of IQGAP3 in 10 GC samples. The bars represent individual samples. Fold-change indicates the ratio of IQGAP3 mRNA expression in GC to that in corresponding nonneoplastic mucosa. **b** Immunohistochemical analysis of IQGAP3 in GC and corresponding nonneoplastic gastric mucosa. Original magnification,

$\times 400$. **c** Immunohistochemical analysis of IQGAP3 in well-differentiated type GC. Original magnification, $\times 400$. **d** Immunohistochemical analysis of IQGAP3 in poorly-differentiated type GC. Original magnification, $\times 400$. **e** Kaplan-Meier plot of survival in patients with GC. **f** Summary of IQGAP3 expression and expression of the mucin phenotype.

The expression of IQGAP3 was scored in all tumors as positive or negative. When more than 10% of tumor cells were stained, immunostaining was considered positive for IQGAP3. Using these definitions, two surgical pathologists (N.O. and K.S.) without knowledge of clinical and pathologic parameters or patient outcome independently reviewed immunoreactivity in each specimen. Interobserver differences were resolved by consensus review at a double-headed microscope after the independent review.

Phenotypic Analysis

GCs were classified into 4 phenotypes: gastric (G) type, intestinal (I) type, gastric and intestinal mixed (GI) type, and null (N) type. For phenotypic expression analysis of GC, we performed immunohistochemical analysis (as described above) with 4 antibodies: anti-MU-

C5AC (Novocastra, Newcastle, UK) as a marker of foveolar epithelial cells in the stomach, anti-MUC6 (Novocastra) as a marker of pyloric gland cells in the stomach, anti-MUC2 (Novocastra) as a marker of goblet cells in the small intestine and colorectum, and anti-CD10 (Novocastra) as a marker of microvilli of absorptive cells in the small intestine and colorectum. The criteria for the classification of G type and I type GCs have been described previously [1]. Briefly, GCs in which more than 10% of cells in the section expressed at least one gastric epithelial cell marker (MUC5AC or MUC6) or intestinal epithelial cell marker (MUC2 or CD10) were classified as G type or I type cancers, respectively. Sections that showed both gastric and intestinal phenotypes were classified as GI type, and those that lacked both the gastric and intestinal phenotypes were classified as N type. The phenotype status has been determined previously [7].

Cell Lines

Four cell lines derived from human GC (MKN-1, MKN-7, MKN-45, and MKN-74) were purchased from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan). All cell lines were maintained in RPMI 1640 (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% fetal bovine serum (BioWhittaker; Lonza, Basel, Switzerland) in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C.

RNA Interference

siRNA oligonucleotides targeting *IQGAP3* and a negative control were purchased from Invitrogen (Thermo Fisher Scientific, Inc.). We used 2 independent *IQGAP3* siRNA oligonucleotide sequences. Transfection was performed using Lipofectamine RNAiMAX (Invitrogen) as described previously [17]. Briefly, 60 pmol of siRNA and 10 µL of Lipofectamine RNAiMAX were mixed in 1 mL of RPMI medium (10 nmol/L final siRNA concentration). After 20 min of incubation, the mixture was added to cells, which were then plated in culture dishes. Cells were analyzed 48 h after transfection.

Western Blot Analysis

Cells were lysed as described previously [18]. Lysates (40 µg) were solubilized in Laemmli sample buffer by boiling and then subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by electrotransfer onto nitrocellulose membranes. Anti-extracellular signal-regulated kinase 1/2 (Erk1/2), anti-phospho-Erk1/2 (pErk1/2), anti-Akt, and anti-phospho-Akt (pAkt) were purchased from Cell Signaling Technology (Danvers, MA, USA). Peroxidase-conjugated anti-mouse or anti-rabbit IgG was used in the secondary reaction. Immunocomplexes were visualized with an ECL Western Blot Detection System (Amersham Biosciences). β-Actin (AC-15; Sigma Chemical, St. Louis, MO, USA) was also stained as a loading control.

Cell Growth Assay

To examine cell growth, we performed 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. The cells were seeded at a density of 2,000 cells per well in 96-well plates. Cell growth was examined after 1, 2, and 4 days. Three independent experiments were performed, and means and standard deviations (SD) were calculated for each experiment.

Spheroid Colony Formation

For the generation of spheres, 2,000 cells were plated per well in 24-well ultra-low attachment plates (Corning; New York, NY, USA). Cells were grown in mTeSR medium (STEMCELL Technologies Inc., Cambridge, MA, USA). The plates were incubated at 37 °C in a 5% CO₂ incubator for 15 days, after which the sphere number and size were determined under a microscope.

Statistical Analysis

Associations between clinicopathologic parameters and *IQGAP3* expression were analyzed by the Fisher exact test. Kaplan-Meier survival curves were constructed for *IQGAP3*-positive and *IQGAP3*-negative patients. Survival rates were compared between *IQGAP3*-positive and *IQGAP3*-negative groups. Differences between survival curves were tested for statistical significance by a log-rank test. Differences in the sphere number and size between the 2 groups were tested by the Student *t* test.

Table 1. Relationships between *IQGAP3* expression and clinicopathologic characteristics

	<i>IQGAP3</i> expression		<i>p</i> value
	positive	negative	
Age			
≤65 years	19 (23%)	65	0.5668
>65 years	15 (19%)	66	
Sex			
Male	21 (21%)	81	1.0000
Female	13 (21%)	50	
T classification			
T1	10 (14%)	62	0.0783
T2/3/4	24 (26%)	69	
N classification			
N0	11 (13%)	74	0.0132
N1/2/3	23 (29%)	57	
M classification			
M0	22 (17%)	111	0.0139
M1	12 (38%)	20	
Stage			
Stage I	10 (12%)	72	0.0117
Stage II/III/IV	24 (29%)	59	
Histologic classification			
Well differentiated	16 (20%)	65	0.8487
Poorly differentiated	18 (21%)	66	
Lymphatic invasion			
Positive	23 (25%)	70	0.1748
Negative	11 (15%)	61	
Vascular invasion			
Positive	18 (25%)	54	0.2476
Negative	16 (17%)	77	

Results

Expression of *IQGAP3* in GC

We previously showed that *IQGAP3* expression was twofold higher in spheroid body-forming GC cells compared with parental cells. Here, we first analyzed *IQGAP3* mRNA expression in 10 GC tissue samples and nonneoplastic mucosa samples by qRT-PCR (Fig. 1a), and observed the overexpression of *IQGAP3* (tumor/nonneoplastic mucosa ratio >2) in 5 (50%) of 10 GC tissue samples.

Next, we performed immunohistochemical analysis of *IQGAP3* in 165 GC tissue samples. In nonneoplastic gastric mucosa, weak staining of *IQGAP3* was observed in the foveolar epithelium. No staining of *IQGAP3* was found in stromal cells. In contrast, GC tissue showed stronger, more extensive staining (Fig. 1b) in both well-differentiated GC (Fig. 1c) and poorly-differentiated GC (Fig. 1d). A total of 34 (21%) GC cases were positive for

Table 2. Univariate and multivariate Cox regression analysis of IQGAP3 expression and survival in gastric cancer

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Age				
≤65 years	1 (Ref.)			
>65 years	1.12 (0.61–2.04)	0.7188		
Sex				
Male	1 (Ref.)			
Female	1.52 (0.81–3.02)	0.1945		
Stage				
I	1 (Ref.)		1 (Ref.)	
II/III/IV	28.40 (8.72–174.37)	0.0001	20.48 (4.89–142.24)	0.0001
Histologic classification				
Well differentiated	1 (Ref.)			
Poorly differentiated	1.51 (0.83–2.81)	0.1817		
Lymphatic invasion				
Negative	1 (Ref.)		1 (Ref.)	
Positive	9.57 (3.85–31.92)	0.0001	1.62 (0.52–6.50)	0.4289
Vascular invasion				
Negative	1 (Ref.)		1 (Ref.)	
Positive	3.63 (1.93–7.20)	0.0001	0.64 (0.42–1.81)	0.6392
IQGAP3 expression				
Negative	1 (Ref.)		1 (Ref.)	
Positive	4.60 (2.47–8.44)	0.0001	3.49 (1.89–6.46)	0.0002

IQGAP3. IQGAP3 staining was mainly observed on the cell membrane. Many GC cases exhibited IQGAP3 staining heterogeneity, with the percentage of IQGAP3-stained GC cells ranging from 0 to 70%. No tendency for IQGAP3 upregulation at the invasive front was observed.

We next examined the relationship between IQGAP3 staining and clinicopathologic characteristics (Table 1). A marginally significant difference was observed between IQGAP3 expression and T classification ($p = 0.0783$). In contrast, IQGAP3 expression was statistically associated with N classification ($p = 0.0132$) and M classification ($p = 0.0139$). GC cases positive for IQGAP3 were found more frequently in stage II/III/IV cases than in stage I cases. These results suggest that IQGAP3 plays an important role in the progression of GC. Furthermore, Kaplan-Meier analysis demonstrated that IQGAP3-positive GC cases had significantly worse survival than IQGAP3-negative GC cases ($p = 0.0001$; Fig. 1e). We next performed univariate and multivariate Cox proportional hazard analyses to evaluate the potential use of IQGAP3 expression as a prognostic classifier (Table 2). In univariate analysis, stage, lymphatic invasion, vascular invasion, and IQGAP3 expression were associated with poor survival.

Table 3. Relationships between IQGAP3 expression and gastric/intestinal phenotype markers

	IQGAP3 expression		<i>p</i> value
	positive	negative	
MUC5AC			
Positive	30 (34%)	57	0.0001
Negative	4 (5%)	74	
MUC6			
Positive	7 (39%)	11	0.0422
Negative	27 (18%)	120	
MUC2			
Positive	8 (20%)	33	0.8417
Negative	26 (21%)	98	
CD10			
Positive	1 (6%)	22	0.0944
Negative	33 (22%)	114	

In the multivariate model, stage and IQGAP3 expression were found to be independent predictors of survival in patients with GC. These results suggest that IQGAP3 is a promising biomarker with which to identify patients with poor prognosis.

Fig. 2. Effect of *IQGAP3* knockdown in gastric cancer (GC) cells. **a** Western blot analysis of *IQGAP3* protein expression in 4 GC cell lines. **b** Western blot analysis of *IQGAP3*, Akt, phospho-Akt (pAkt), Erk1/2, and phospho-Erk1/2 (pErk1/2) in cell lysates from MKN-74 cells transfected with *IQGAP3* siRNA or negative control siRNA. β -Actin was measured as a loading control. **c** Cell growth was assessed by MTT assays at days 1, 2, and 4 after seeding in 96-well plates. Bars and error bars indicate the mean and SD, respectively, of 3 independent experiments. * $p < 0.05$. **d** The number of spheres from MKN-74 cells transfected with *IQGAP3* siRNA or negative control siRNA. Bars and error bars indicate the mean and SD, respectively, of 3 independent experiments. * $p < 0.05$. **e** The size of spheres from MKN-74 cells transfected with *IQGAP3* siRNA or negative control siRNA. Bars and error bars indicate the mean and SD, respectively, of 3 independent experiments. * $p < 0.05$.

Association between *IQGAP3* Expression and Gastric/Intestinal Phenotype Marker

We investigated associations between *IQGAP3* expression and the mucin phenotype (G type, I type, GI type, and N type). Immunohistochemical analysis of gastric (MUC5AC and MUC6) and intestinal (MUC2 and CD10) markers was used to phenotypically classify the 165 GC cases as G type ($n = 69$, 42%), I type ($n = 27$, 16%), GI type ($n = 26$, 16%), and N type ($n = 43$, 26%). *IQGAP3* was shown to be frequently expressed in MUC5AC-positive and MUC6-positive GC cases (Table 3), and was ob-

served significantly more frequently in GC exhibiting the G phenotype (G type GC plus GI type GC) than in other GC types (I type GC plus N type GC) ($p = 0.0001$, Fisher exact test; Fig. 1f).

Effect of Inhibition of *IQGAP3* on Sphere Number and Size

Our results showed that *IQGAP3* was frequently expressed in GC cases, and GC cases positive for *IQGAP3* were found more frequently in stage II/III/IV cases than in stage I cases. However, the significance of *IQGAP3* ex-

pression in GC remains unclear. Therefore, we next investigated the effect of IQGAP3 inhibition on sphere number and size on GC cells using siRNA.

First, we performed Western blot analysis of IQGAP3 in 4 GC cell lines. IQGAP3 protein expression was detected in MKN-45 and MKN-74 GC cells (Fig. 2a), so these 2 cell lines were selected for the following assays. Western blot analysis revealed that the expression of IQGAP3 in MKN-74 cells was substantially suppressed by treatment with siRNA1 and siRNA2 (Fig. 2b), while MTT assays showed that IQGAP3 siRNA1- and siRNA2-transfected MKN-74 cells had significantly reduced cell growth relative to negative control siRNA-transfected cells (Fig. 2c). We also obtained similar results from an MTT assay performed 4 days after siRNA transfection of MKN-45 cells (data not shown). These results suggest that IQGAP3 is involved in GC cell growth.

The effect of *IQGAP3* inhibition on sphere number and size was also examined in MKN-74 cells. Both the number (Fig. 2d) and size (Fig. 2e) of spheres formed by MKN-74 cells was significantly reduced in *IQGAP3* siRNA1- and siRNA2-transfected cells compared with negative control siRNA-transfected cells. Similar results were obtained for spheres formed by MKN-45 cells (data not shown). These results suggest that *IQGAP3* plays an important role in spheroid formation.

Effect of IQGAP3 Inhibition on Akt and Erk Pathways

Akt and Erk pathways are major signaling pathways that promote cancer cell proliferation [19], while *IQGAP3* is known to interact with Erk1 and enhance its phosphorylation [9, 14]. We therefore examined the effect of *IQGAP3* inhibition on Akt and the Erk pathways in MKN-74 cells. Western blot analysis revealed that the levels of phosphorylated Akt and Erk1/2 were lower in *IQGAP3* siRNA-transfected cells than in negative control siRNA-transfected cells (Fig. 2b). Similar results were observed in MKN-45 cells (data not shown).

Discussion

Previously, we identified several genes that were up-regulated in spheroid body-forming GC cells compared with parental cells [6]. Among these, we reported the up-regulation of *KIFC1* and *KIF11* proteins in GC cases [6, 7], and showed that both the number and size of spheres from GC cell lines were significantly reduced by knockdown of *KIFC1* and *KIF11*. However, these 2 proteins are localized in the nucleus. In the present study, we focused

on transmembrane proteins because the genes that encode them are potentially useful therapeutic targets or biomarkers for cancer diagnosis [8]. We found that *IQGAP3* was upregulated in 21% of GC cases. A marginally significant difference was observed between *IQGAP3* expression and T classification ($p = 0.0783$). Previously, it has been reported that *IQGAP3* silencing in breast cancer cells repressed cell migration and invasion [20]. Thus, there is a possibility that *IQGAP3* may stimulate GC cell invasion. *IQGAP3* expression was statistically associated with N classification ($p = 0.0132$) and M classification ($p = 0.0139$). GC cases positive for *IQGAP3* were found more frequently in stage II/III/IV cases than in stage I cases. Taken together, these results suggest that *IQGAP3* plays an important role in the progression of GC. Furthermore, *IQGAP3* expression was associated with survival, and served as an independent prognostic classifier of patients with GC. These results suggest that *IQGAP3* immunostaining is a clinically useful method for the prediction of GC patient survival.

In the present study, MTT assays demonstrated that *IQGAP3* knockdown inhibited cell growth. Although the underlying mechanisms remain unclear, we showed that the levels of phosphorylated Akt and Erk were lower in *IQGAP3* siRNA-transfected GC cells than in negative control siRNA-transfected cells. *IQGAP3* has previously been reported to interact with Erk1 and to enhance Erk1 phosphorylation following treatment with epidermal growth factor [9]. Because Erk signaling induces cell proliferation [19], it is likely to play an important role in promoting GC cell growth through interaction with *IQGAP3*. In contrast, the association between Akt signaling and *IQGAP3* has not yet been examined, although the knockdown of *IQGAP1* was shown to suppress the activation of Akt by human epidermal growth factor receptor 2. Additionally, the activation of Akt in mouse cardiac tissue induced by chronic pressure overload was not detected in *IQGAP1*-null mice [21]. Although it is conceivable that *IQGAP3* also activates Akt signaling, further work is required to clarify this.

CSCs are defined as a unique subpopulation of cells that possess self-renewal and differentiation potential, and that are generally responsible for tumor initiation, invasion, metastasis, and chemo-resistance [22]. Thus, CSC characterization can improve our understanding of carcinogenesis and help explain the frequency of treatment failure for GC. In the present study, we showed that knockdown of *IQGAP3* inhibited spheroid formation of GC cells. Previously, the Akt and Erk signaling pathways were shown to be essential for maintaining the pluripo-

tency of stem cells [23, 24]. Moreover, gastric spheroid cells are known to overexpress multiple stem cell markers, including Oct4, Sox2, Gli1, CD44, CD133, pAkt, and pErk [22]. Taken together, these results suggest that the phosphorylation of Akt and Erk by IQGAP3 is involved in spheroid formation by GC cells. However, CSCs are a minority population (<5%) of cells [25], and we showed here that IQGAP3 is not a specific marker for gastric CSCs because the percentage of IQGAP3-stained GC cells ranged from 0 to 70%.

The overexpression of IQGAP3 has been reported in several human cancers including hepatocellular carcinoma, lung cancer, pancreatic cancer, and breast cancer [9, 20, 26, 27]. *IQGAP3* is located at 1q21.3, which is a hotspot for gene amplification in cancer [28]. DNA amplification at 1q21.3 has previously been reported in GC [29], so 1q21.3 amplification may contribute to the up-regulation of *IQGAP3*. Future work should examine the association between 1q21.3 amplification and *IQGAP3* expression.

In summary, we demonstrated that *IQGAP3* is overexpressed in GC, and that *IQGAP3* expression is an independent prognostic classifier of patients with GC. We also showed that knockdown of *IQGAP3* inhibited cell growth and spheroid formation. Because *IQGAP3* is a transmembrane protein, antibodies against *IQGAP3* may constitute promising anticancer drugs.

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Disclosure Statement

The authors declare no conflict of interest.

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