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The Generation and Application of Monoclonal Antibodies to Detect SAPCD2 Expression in Precancerous and Malignant Gastric Lesions

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ABSTRACT

Background: Suppressor APC domain containing 2 (SAPCD2) is involved in cell cycle regulation and its mRNA levels are higher in cancer tissues. But, the function of SAPCD2 in cancer development remains unclear.

Objective: To generate mouse monoclonal antibodies (mAbs) specific to SAPCD2 and thus clarify the function of SAPCD2 in the development of gastric carcinoma (GC).

Methods: Purified SAPCD2-GST immunized BALB/c mouse spleen cells were collected and fused with myeloma cells to obtain monoclonal antibody hybridoma. A group of monoclonal antibodies exhibiting high specificity and sensitivity against SAPCD2 has been generated and characterized by IHC, WB, IP, IF, and ELISA. By immunohistochemical analysis, the SAPCD2 expression was evaluated in 228 clinical samples of gastric mucosal lesions, including precancerous lesions and GC samples.

Results: We identified a highly specific and sensitive clone of s12 in eukaryotic cells and performed an IHC analysis. We found that 81.3% of 107 GC tissues were SAPCD2-positive, compared with the 26.2% in the matched adjacent normal-appearing tissues ($P<0.001$). Furthermore, among the 121 gastritis tissues, SAPCD2 was overexpressed in precancerous gastric lesions such as dysplasia (Dys, 78.9%), intestinal metaplasia (IM, 44.7%), and chronic atrophic gastritis (CAG, 6.1%) compared with that in chronic non-atrophic gastritis (CNAG, 3.2%) ($P<0.001$). The SAPCD2-positivity rate was 81.3% in GC, suggesting that the expression of SAPCD2 increased with the severity of the lesion ($P<0.001$).

Conclusion: In summary, we have described novel monoclonal antibodies against SAPCD2, which are highly expressed in GC tissues and may serve as the basis for an early clinical marker for GC development.

Keywords: Gastric Cancer; Monoclonal Antibody; Precancerous Gastric Lesions; SAPCD2

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INTRODUCTION

Though the incidence of gastric cancer (GC) is declining, it still remains the fifth most common malignancy with a poor prognosis and the third cancer for mortality worldwide (1-4). GC occurs at very high frequency in Eastern Asia including China (3, 5, 6) and its prevention is one of the most important interests of cancer research. According to epidemiology data, Helicobacter pylori infection, salty diet, smoking, and heavy alcohol consumption play important roles in the high incidence and mortality rates of GC (7, 8), and preventing these potential risk factors is helpful for reducing its incidence. A cascade of precursor lesions, such as dysplasia (Dys), intestinal metaplasia (IM), and chronic atrophic gastritis (CAG), precede the intestinal type of GC (9). Gastric carcinomas are detected at an early and curable stage by screening and surveillance, and endoscopy is considered an effective surveillance method for GC with the advantages of direct visualization of gastric mucosa and the collection of mucosal specimens for histopathological evaluation (10, 11). However, endoscopy is an invasive procedure with potential complications. In low-risk populations and regions with low incidences of GC, such as Western Europe and North America, mass screening by endoscopy is not the most popular practical approach, except in Korea and Japan (12). Therefore, identifying molecular markers of early phases of GC as surveillance tools, may greatly improve its diagnosis and treatment.

We previously found that suppressor APC domain containing 2 (SAPCD2) is consistently expressed in diverse embryonic tissues and GC tissues, but not in the matched normal mucosa of the stomach, using a polyclonal antibody (13). Moreover, studies showed that its expression was cell cycle-dependent, and the depletion of the SAPCD2 expression significantly suppressed cell proliferation and tumorigenicity (14, 15). Furthermore, another study showed that the mRNA level of SAPCD2 significantly elevated in colorectal cancer (16). These findings indicate that SAPCD2 plays an important role in tumorigenesis. However, the accuracy, specificity, and stability of monoclonal antibodies (mAbs) will affect the extensive evaluation of the significance of SAPCD2 in both basic and clinical studies, including the characterization of its role in different stages of the GC progression.

In the current work, we generated and characterized 13 mAbs against SAPCD2 by enzyme-linked immunosorbent assay (ELISA), western blot (WB), immunoprecipitation (IP), immunohistochemistry (IHC), and immunofluorescence (IF) analysis. Our recently discovered SAPCD2 monoclonal antibodies facilitated the identification of the SAPCD2 expression in GC and gastritis tissues through IHC, as well as in GC cell lines via RT-PCR and WB, thus providing a valuable tool for investigating the SAPCD2's potential role as an early clinical marker in gastric cancer development.

MATERIALS AND METHODS

Patients' Characteristics

We collected 228 patient samples including gastritis and GC (Table 1). 107 samples from the patients with GC treated at Huadu District People's Hospital from 2014 to 2018 were assessed, including 76 males (71%) and 31 females (29%). Matched adjacent normal-appearing tissues (NAT, at least 5 cm away from the edge of the tumor) of GC were collected. Gastritis samples were collected by endoscopy from 121 patients including 47 men (38.8%) and 74 women (61.2%). All the GC patients underwent radical resection with curative intent. GC and gastritis were evaluated histologically by a senior specialist in the Department of Pathology.

Cell Culture

BGC823, MGC803, SGC7901, AGS, N87, GES1, NIH3T3, and SP2/0 cell lines were procured from the Institute of Biochemistry and Cell Biology, Chinese Academy of

Table 1. Clinical and pathologic characteristics of 228 patients samples used in IHC analysis

Tissues parameters	Number (%)
Gastric cancer tissue	107
Gender	
Male	76 (71%)
Female	31 (29%)
Age	
Male	(19-87)
Female	(29-88)
Pathology	
Intestinal	100 (93.5%)
Diffuse	4 (3.7%)
Mixed	3 (2.8%)
Differentiation	
Well	5 (4.7%)
Moderate	28 (26.2%)
Poor	72 (67.3%)
TNM stage	
I	15 (14.0%)
II	40 (37.4%)
III	35 (32.7%)
IV	17 (15.9%)
Gastritis and precancerous lesions	121
Gender	
Male	47 (38.8%)
Female	74 (61.2%)
Age	
Male	21-78
Female	15-73
Pathology	
Dys	19 (15.7%)
IM	38 (31.4%)
CAG	33 (27.3%)
CNAG	31 (25.6%)

Tumour, node and metastasis (TNM), Intestinal metaplasia (IM), Chronic atrophic gastritis (CAG), Chronic non-atrophic gastritis (CNAG)

Sciences (Shanghai, China). And BGC823, MGC803, SGC7901, AGS, N87, and GES1 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, NY, US). For AGS and N87, DMEM was supplemented with 10% fetal bovine serum (Gibco, NY, US); culture medium for BGC823, MGC803, SGC7901, and GES1 was supplemented with 5% FBS; and NIH3T3 cells were cultured in DMEM supplemented with 10% FBS (Hyclone, Logan, US). Mouse myeloma cells SP2/0 were cultured in RPMI 1640 medium (Hyclone, Logan, US) supplemented with 10% FBS. All the cell lines were cultured at 37°C with 5% CO_2 and a certain humidity.

Expression of Recombinant SAPCD2-GST Fusion Proteins in Escherichia Coli

We constructed SAPCD2 expression vector based on the Homo sapiens gene for tumor specificity and mitosis phase-dependent expression protein mRNA in GenBank (DQ150361). Next, we extracted the total RNA from BGC823 cells using Trizol reagent (Invitrogen, CA, US), and prepared cDNA using the EasyScript First-Strand cDNA Synthesis Supermix (AE301, Ttransgen, Beijing, China) following the manufacturer's instructions. Using the synthesized cDNA as a template and two primers, we amplified the full-length coding region via PCR. The two primers used were 5'-TGAGGATCCGGTGGACGAGGG-3', which included the sequences 87-98 in SAPCD2 mRNA and an additional BamHIsite, and 5'-ATGAAGC TTGAAGGGCTGAGTGCCAG-3', which included the complementary sequences of 1335-1351 in SAPCD2 mRNA and an additional Hind III site. Following this, we purified the PCR products and inserted them into a modified prokaryotic expression system pET30a-GST after the restriction endonucleases digest. The resulting ligation products were then transformed into Escherichia coli BL21 competent cells, and the recombinant DNA was digested by Bam HI and Hind III, and verified by DNA sequencing. Finally, we used the QIAquick DNA Purification kit (Qiagen, Dusseldorf, Germany) for DNA purification.

To prepare the SAPCD2-GST fusion protein, we cultured the transformed bacteria in an LB medium with kanamycin (30 μg/ml; HARVEYBIO, Beijing, China). Next, we induced the expression of the fusion protein by adding isopropyl β-D-1-thiogalactopyranoside (IPTG, 0.2 mM) to the culture and then it was shaken at 37 °C for 3 h at a stable

temperature. We , then, collected the bacteria by centrifugation, then resuspended them in phosphate-buffered saline (PBS) containing 1 mM phenylmethanesulfonyl fluoride, 1 mg/ml lysozyme, and 1% Triton X-100 to lyse the cells and, finally, release the protein. We used sonication to clarify the bacterial suspension, followed by centrifugation at 12,000 g for 10 min at 4 ℃ to remove cell debris and obtain the crude extract. We then purified the SAPCD2-GST fusion protein from the crude extract using glutathione beads following the manufacturer's instructions (Amersham Biosciences, Piscataway, US). We next analyzed the purified SAPCD2-GST fusion protein by running 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) to determine its size and purity.

Antibody Preparation

BALB/c mice aged 6-8 weeks were selected for primary immunization by subcutaneous multipoint injection of 50 μg SAPCD2-GST. The immunization cycle was done every three weeks, with a total of three immunizations, and the last shock immunization was administered 3 days before the fusion. Antibody titers were measured by ELISA during the first week after immunization, and the serum samples were selected as the negative control before immunization. Subsequent cell fusion was carried out according to the experimental results.

Cell Fusion and Culture

The preparation of hybridomas in cell fusion is based on the technique of Kohler and Milstein (17). The spleen was removed aseptically and a splenic cell suspension was prepared, mixed with the prepared homogenic myeloma cells in a ratio of 10:1, and 50% fusion promoter polyethylene glycol 4000 (Merck, Darmstadt, Germany) was added. The fusion hybridoma cells were screened using HAT (hypoxanthine, aminohysteroside, and thymine; Sigma, St. Louis, US) selected medium for 15 days. Hybridoma cells produce and secrete monoclonal antibodies, and 480 clones of cell culture supernatant were collected and screened by ELISA. After screening positive hybridoma cells that could produce specific monoclonal antibodies, we cloned and amplified them in time, and the clones were isotyped using a commercial kit (Sigma, St. Louis, US).

ELISA

The GST or the SAPCD2-GST fusion protein was diluted in a coated solution (pH 9.5) to a concentration of 5μg/ml. 50 μl of the diluted liquid was added to the labeled plate and coated overnight. After washing it, we added 5% skim milk to each well and sealed them for 2 h at 37 ℃. The supernatant collected from the hybridoma culture was added into 96-well plates with 100 microliters per well, incubated at room temperature for 1 h, and then cleaned with 1×PBST washing solution several times. The supernatant collected from the hybridoma culture was added into 96-well plates with 100 microliters per well, incubated at room temperature for 1 h, and cleaned with 1×PBST washing solution several times. Then we added suitable enzyme-labeled secondary antibodies for incubation and washed them again. Once washed, the substrate solution diluted with citrate phosphate buffer (pH 5.0) was added for chromogenic reaction, and the reaction was carried out for 10 min under dark conditions. At the end of the chromogenic reaction, 50 μl termination solution (12.5% H_2SO_4) was added to each well to terminate the reaction. The OD value of 492 nm was read by a microplate absorbance reader (Model 550, Bio-Rad, Hercules, US), and the antibody level in the hybridoma supernatant culture medium was analyzed according to the value.

Western Blotting (WB) and Immunoprecipitation (IP)

Also, the effectiveness of monoclonal antibodies specifically recognizing SAPCD2 was again verified by WB assay. Total proteins were extracted from the SAPCD2-GST fusion protein, BGC823, and NIH3T3 cells, and 12%

separation gel was used for polyacrylamide gel electrophoresis (PAGE). The proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA, US) activated with methanol, and the PVDF membrane was placed in a blocking solution at room temperature for 1 h. The blocking solution was 5% skim milk diluted with PBST. After sealing, the diluted hybridoma culture supernatant (1:200) was added directly and incubated at 4°C overnight. After washing them three times, an appropriate HRP-coupled secondary antibody (1:5000) was added for incubation. Finally, we used a chemiluminescence apparatus (Tanon, Shanghai, China) to visualize the bands.

For the IP experiment, we also first extracted total proteins from BGC823 cells with IP lysate. We added 10 µl s12 monoclonal antibodies to 200 µg protein solution and incubated them overnight with slow shaking at 4°C. Next, we added the pretreated protein G/A agarose beads and incubated them for 2 h to help the antibody conjugate agarose beads. The agarose beads were eluted several times with PBS buffers, then the sample buffer was added to the eluent. The protein interaction was determined by WB analysis.

Plasmid Construction and Cell Transfection

We cloned the entire coding region of SAPCD2 into the pIRESneo vector (donated by Dr. Min Zhu, Beijing Cancer Hospital) at the Hind III and BamH I sites. Transfection was performed after sequencing verification. NIH3T3 cells were cultured, and the cell culture medium of 2×10^5 cells per well was added to the cell culture plate until the cell density reached 40%-60%. Dilute Lipofectamine 2000 (Invitrogen, California, US) and plasmid DNA were mixed for cell transfection, and the fresh medium was replaced 6 h later. To obtain stably transfected cells, cell passage, and screening were performed 24 h after transfection, and G418 (200 µg/ml; HARVEYBIO, Beijing, China) was used for 21 consecutive days. Subsequently, WB was used to identify the effect of overexpression.

Confocal Immunofluorescence Microscopy

BGC823 cells were cultured to the logarithmic phase and plated on cell slides at a relatively low density until the second day, then fixed in 90% methanol in MeS Buffer for 5 min at 4°C. The cells were subsequently washed thoroughly with PBS and blocked. SAPCD2 monoclonal antibody s12 (1:1500) was used for the incubation of the primary antibody, goat anti-mouse IgG (1:200; Zhong Shan Co., Beijing, China) was used for the secondary antibody, and the nuclei were stained with DAPI (Zhong Shan Co., Beijing, China) for 5 min after washing with PBS. After the seal was dried, the experimental observation was carried out by Leica TCS SP5 confocal microscope (Leica, Heidelberg, Germany).

IHC Analysis

For IHC studies, the paraffin-embedded tissue blocks were sliced with a thickness of 5 µm, dewaxed and rehydrated, and then incubated with 3% hydrogen peroxide solution for 10 min at RT (18). After antigen repair, the paraffin sections were sealed and 5% skim milk was used as a sealing solution. Subsequently, the sections were incubated with the antibody and firstly incubated with SAPCD2 monoclonal antibody s12 (1:1500) at 4 °C overnight. After washing with PBS buffer solution, the labeled secondary antibody (Dako Cytomation, Cambridgeshire, UK) of horseradish peroxidase was incubated at room temperature for 30 min. Color development was observed with diaminobenzidine (DAB; Sigma, St. Louis, US) for 5 min, and then reverse staining was performed with hematoxylin. Purified IgG from the serum of normal mice was selected as a negative control for the comparative study. The count of tumor cells and normal gastric glandular epithelial cells was performed by two specialists in the Department of Pathology, and the criterion for determining positive cases was more than 20% immunostained cells.

Statistical Analysis

The statistical analysis was performed

using SPSS 20.0 (SPSS, Inc., Chicago, IL). Pearson's χ^2 test was utilized to evaluate potential differences in the SAPCD2 expression across various gastritis and GC specimens. A p-value less than 0.05 was considered statistically significant.

RESULTS

Preparation of the SAPCD2-GST Fusion Protein and Development of mAbs

We cloned the CDS region (Figure 1A) of the SAPCD2 gene and expressed the

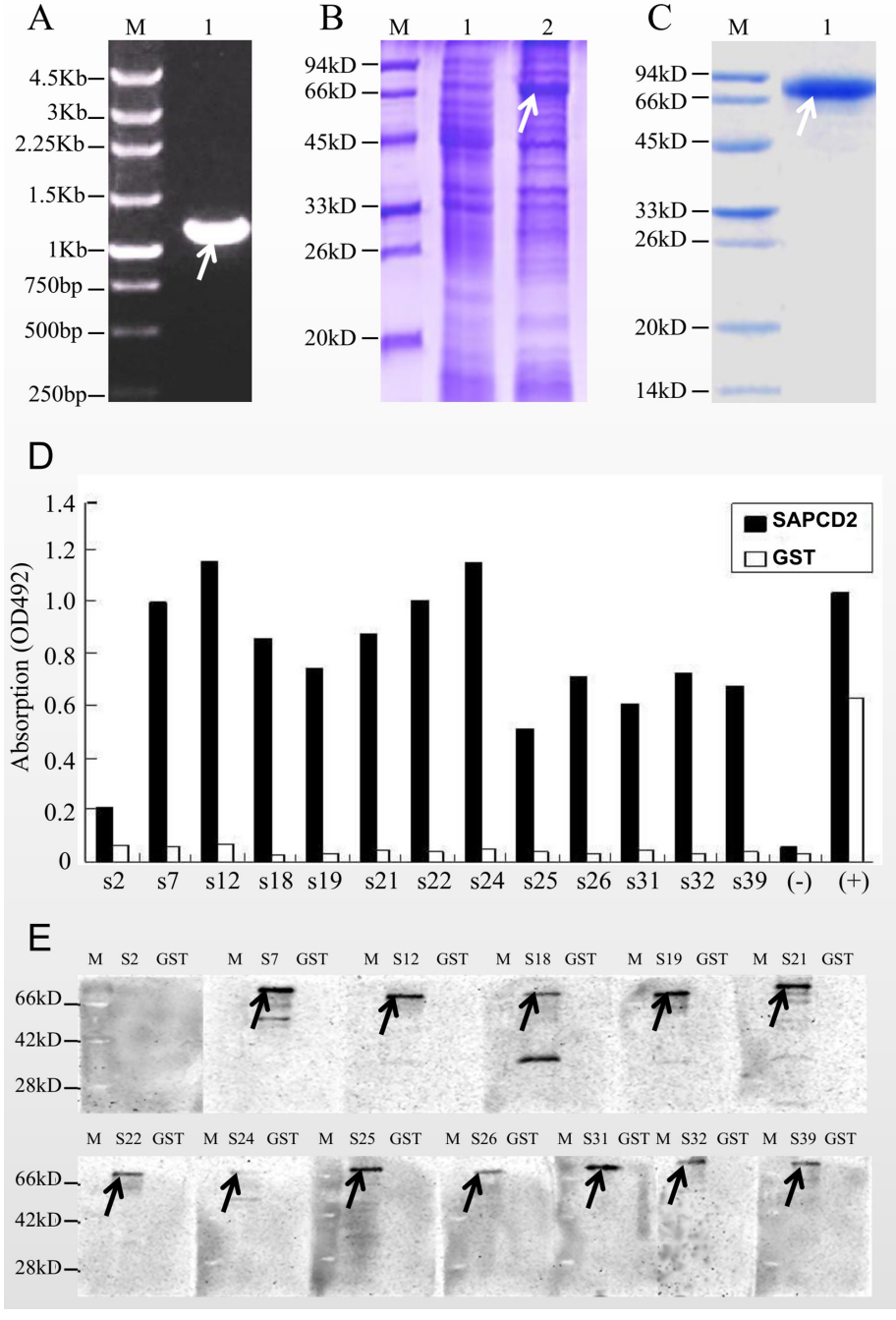

Figure 1. Preparation of SAPCD2-GST fusion protein and development of mAbs. The arrow points to recombinant SAPCD2-GST protein (B, C, E). A: CDS region of SAPCD2; B: expression of SAPCD2 in *E. coli* before and after SAPCD2-GST induction in lane 1 and lane 2, respectively; C: the fusion protein, SAPCD2-GST was purified with glutathione beads and examined by SDS-PAGE; D: 13 hybridomas clones (s2, s7, s12, s18, s19, s21, s22, s24, s25, s29, s31, s32, s39) were analyzed by ELISA to compare specificities to SAPCD2 and GST. E: All 13 hybridomas clones recognized recombinant SAPCD2-GST protein, except clone s2; no clone reacted with GST protein. glutathione S-transferase (GST) ,Suppressor APC domain containing 2 (SAPCD2)

SAPCD2-GST (Figure 1B) in E. coli. Then the fusion protein SAPCD2-GST was recycled from the soluble portion of E. coli lysate and further purified with glutathione beads (Figure 1C). The apparent molecular weight of the purified protein was 68 kDa by SDS-PAGE analysis.

The spleen cells of BALB/c mice immunized with the purified SAPCD2-GST were collected and fused with myeloma cells to obtain mAbs hybridomas, as described in Section 2. We screened all 480 clones of surviving hybridomas by ELISA and compared their specificities for SAPCD2 and GST and identified 13 hybridomas clones (s2, s7, s12, s18, s19, s21, s22, s24, s25, s29, s31, s32, s39) that specifically recognized SAPCD2 but did not react with GST (Figure 1D).

To further confirm the specificity of these 13 SAPCD2 mAbs, we performed WB analysis using purified GST or total protein obtained from bacteria transformed with SAPCD2-GST (Figure 1E). Similar to the ELISA data, all hybridomas clones recognized the recombinant SAPCD2-GST protein purified from the prokaryotic cells except clone s2; none of them reacted with GST protein. Four of the clones (s12, s22, s31, and s39) were more specific, given their distinct bands with no other nonspecific bands. All the characterization and isotypes of 13 mAbs are shown in Table 2.

Characterization of s12 mAb of SAPCD2

A single highly immunoreactive band was detected at around 43 kDa with s12 mAb. Following stable transfection to overexpress SAPCD2 in NIH3T3 cells, the immunoreactivity of s12 with endogenous SAPCD2 was further validated by WB. The expression of exogenous SAPCD2 mRNA was confirmed by RT-PCR (Figure 2A). Enhanced detection of endogenous SAPCD2 in NIH3T3 cells also confirmed the specificity of s12 mAb. Furthermore, s12 mAb interacted with the native SAPCD2 protein from BGC823, MGC803, SGC7901, AGS, N87, and GES1 cells (Figure 2B), indicating that SAPCD2 is expressed in all of these cell lines.

To test whether s12 mAb recognizes the native SAPCD2 protein in non-denatured

Table 2. Specific characterization and isotype of mAbs to SAPCD2

Clone	SAPCD2 expressed in E. coli					isotype
	ELISA		WB			
	SAPCD2	GST	SAPCD2	GST	Non-specific band	
S2	+	-	-	-	-	IgG2b
S7	+	-	+	-	+	IgG2a
S12	+	-	+	-	-	IgG1
S18	+	-	+	-	+	IgG1
S19	+	-	+	-	+	IgG1
S21	+	-	+	-	+	IgG1
S22	+	-	+	-	-	IgG1
S24	+	-	+	-	+	IgG1
S25	+	-	+	-	+	IgA
S29	+	-	+	-	+	IgG1
S31	+	-	+	-	-	IgG2b
S32	+	-	+	-	+	IgG1
S39	+	-	+	-	-	IgG1

For ELISA, the magnitudes of immunoreactivity to SAPCD2 were compared with the signals generated by antibody against GST. + indicates OD492 scanning signal versus anti-GST. For WB, + indicates the monoclonal antibody could interact with SAPCD2 and have non-specific immunoreactivity band in column SAPCD2 and non-specific band, respectively. The characterization of subclasses of SAPCD2 mAbs was performed with mouse monoclonal antibody isotyping kit (Sigma). Glutathione S-transferase (GST); Enzyme-linked immunosorbent assay (ELISA); Western Blotting (WB); Suppressor APC domain containing 2 (SAPCD2)

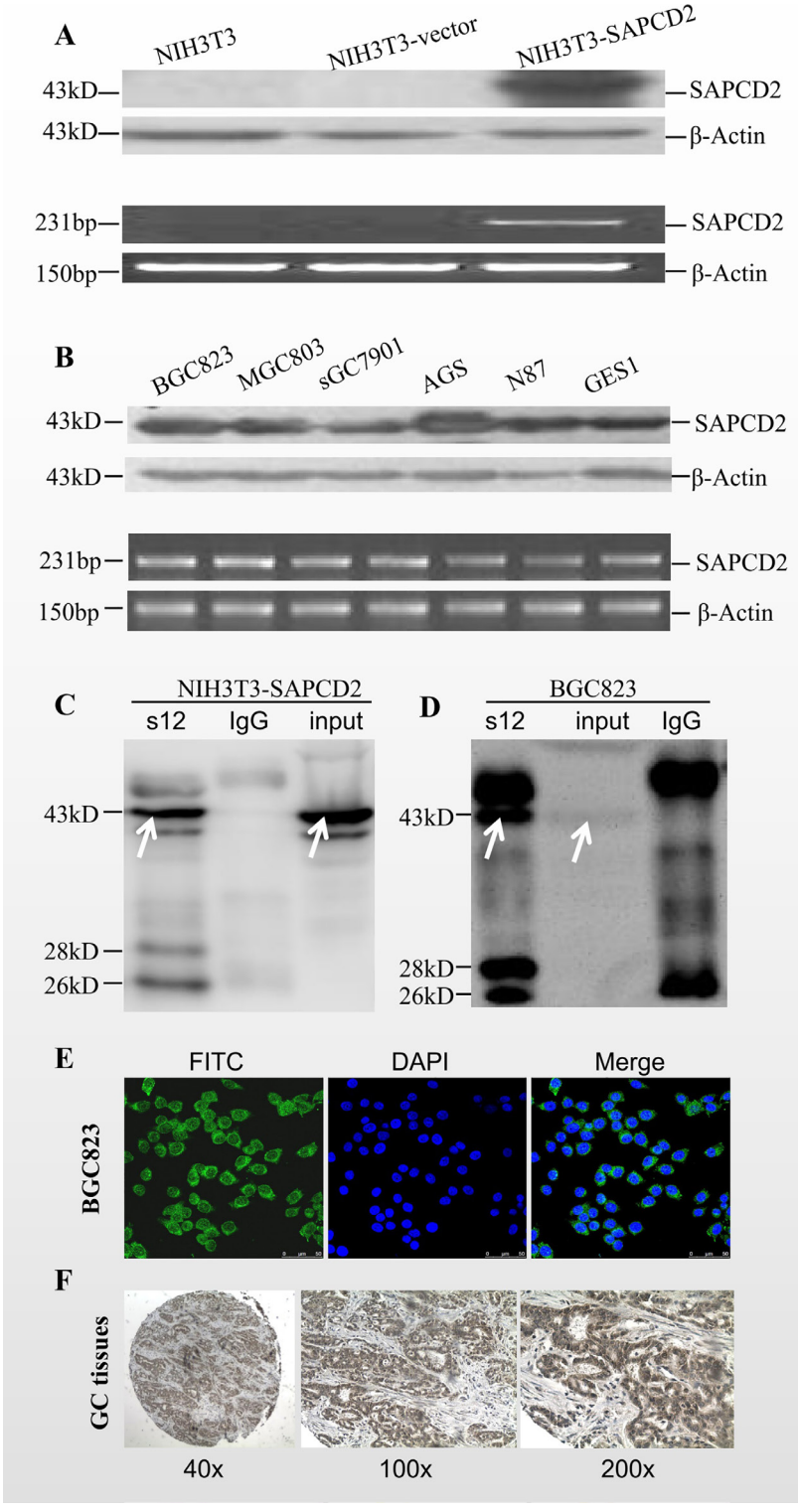

Figure 2. Application of s12 mAb in identification of endogenous SAPCD2 protein. S12 mAb recognized native and enhanced endogenous SAPCD2 protein in non-denatured condition, and confirmed SAPCD2 localization in cells. A: stable transfection with SAPCD2 mRNA in NIH3T3 cells, transfection was validated by RT-PCR and immunoreactivity of s12 with endogenous SAPCD2 was demonstrated by WB; untransfected NIH3T3 and NIH3T3-vector are transfection controls; β-actin used as a loading control. B: s12 mAb interacted with native SAPCD2 protein of BGC823, MGC803, SGC7901, AGS, N87, and GES1 cells. S12 mAb interacted with over-expressed and native SAPCD2 protein in NIH3T3-SAPCD2 cells (C) and BGC823 (D) as confirmed by IP, IgG and input protein as control. IF and IHC assay was performed to demonstrate that SAPCD2 localized predominantly in the cytoplasm of BGC823 (E) and GC (F) cells. Suppressor APC domain containing 2 (SAPCD2), Gastric cancer (GC), 4',6-diamidino-2-phenylindole (DAPI)

conditions and assess the location of the protein in cells, IP, IF, and IHC assays were performed. As shown, s12 interacted with the non-denatured SAPCD2 protein from BGC823 and NIH3T3-SAPCD2 cells by IP (Figure 2C, 2D). Confocal microscopy and IHC analysis confirmed that SAPCD2 is localized predominantly in the cytoplasm of BGC823 and GC cells (Figure s 2E, 2F).

Expression of SAPCD2 in Gastritis and GC Tissues

The expression of SAPCD2 in gastritis and GC tissues was checked by IHC analysis using s12 mAb. As shown in Figure 3, SAPCD2 was chiefly dyed in the cytoplasm of GC cells consistent with the confocal microscopy results in cell lines. Moreover, we observed a considerable difference in the expression of SAPCD2 among 107 GC tissues with matched adjacent NAT; their SAPCD2-positivity rates were 81.3% and 26.2%, respectively (P<0.001) (Table 3). In 121 gastritis tissues, SAPCD2 was overexpressed in precancerous gastric lesions such as Dys, IM, and CAG compared with the CNAG; the SAPCD2-positivity rates were 78.9% (Dys), 44.7% (IM), 6.1% (CAG), 3.2% (CNAG) (P<0.001), respectively. Considering that SAPCD2 was expressed in 81.3% of the GC tissues examined, the expression of SAPCD2 seemed to gradually increase with the severity of the lesion (P<0.001, linear-by-linear association) (Table 4). These results suggest that SAPCD2 may be an early clinical marker for the development of GC.

DISCUSSION

SAPCD2 was identified more than ten years ago as p42.3 or Corf140; and has been implicated as an important player in tumorigenesis by some investigations (13). However, the function of SAPCD2 in the process of tumorigenesis is still unclear, largely because of the accuracy, specificity, and stability of the monoclonal antibodies. Therefore, we generated and characterized a cluster of mAbs with manifest specificity and sensitivity against SAPCD2 using ELISA, WB, IP, IF, and IHC. We evaluated the expression of SAPCD2 in 228

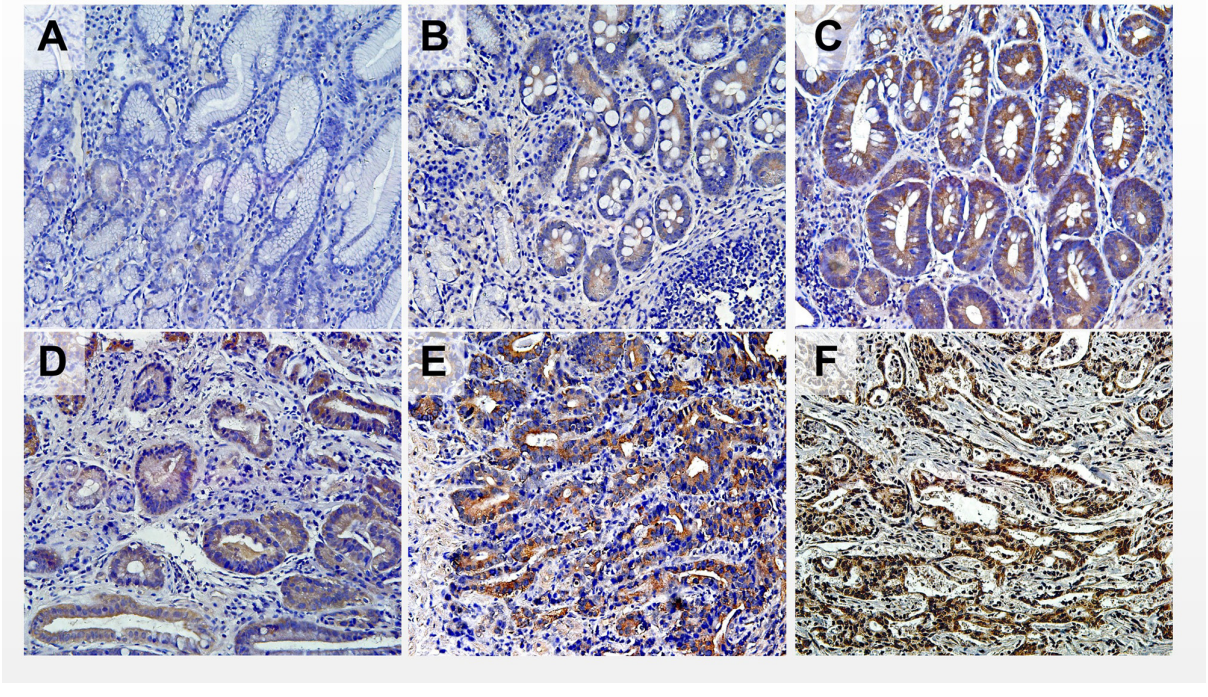

Figure 3. Expression of SAPCD2 in gastritis and GC tissues. Expression of SAPCD2 in gastritis and GC tissues by IHC analysis was examined by IHC using s12 mAb. A, low expression level of SAPCD2 is observed in CNAG. SAPCD2 is overexpressed in IM (B, C), Dys (D, E), and GC (F).

Table 3. Expression of SAPCD2 between GC and adjacent normal gastric tissues Expression of SAPCD2 on gastric specimens

Type	Cases	Positive	Negative	χ^2	P value
GC	107	87 (81.3)	20 (18.7)	65.431	<0.001
NAT	107	28 (26.2)	79 (73.8)		

Normal appearing tissue (NAT); Gastric cancer (GC)

Table 4. Expression of SAPCD2 in gastric specimens

Type	Cases	SAPCD2 expression (%)		P value
		Positive	Negative	
All patients	228			<0.001[a]
Cancer	107	87 (81.3)	20 (18.7)	<0.001[b]
Dys	19	15 (78.9)	4 (21.1)	
IM	38	17 (44.7)	21 (55.3)	
CAG	33	2 (6.1)	31 (93.9)	
CNAG	31	1 (3.2)	29 (96.8)	

[a]Pearson Chi-Square; [b]Linear-by-Linear Association; Intestinal metaplasia (IM), Chronic atrophic gastritis (CAG), Chronic non-atrophic gastritis (CNAG)

clinical samples of gastric mucosal lesions, including precancerous lesions and GC samples, by IHC analysis.

All 13 mAbs identified, except s2, show high specificity and sensitivity to the SAPCD2-GST fusion protein but not GST protein in ELISA and WB analyses. Our findings indicated that 4 clones (s12, s22, s31, and s39) have more specific bands on WB and would be more suitable tools for future research. Furthermore, s12 mAb interacted well with endogenous SAPCD2 in GC cells by WB and IP; it also had a strong reaction to SAPCD2 in IHC analysis. Thus, s12 mAb was used for further characterization of the expression and localization of SAPCD2 by WB, IP, IF, and IHC.

In this study, we found that SAPCD2 is expressed in all 6 gastric cell lines at mRNA and protein levels. Examination of the SAPCD2 expression in 107 GC tissues and matched NAT, as well as in 121 gastritis tissues by the IHC using s12 mAb demonstrated that the SAPCD2 expression level was higher in GC tissues than in the NAT (81.3% vs 26.2%, p<0.001). Furthermore, the data shows the expression ratio was only 3.2% for patients with CNAG, but the expression ratio rose to 6.1%, 44.7%, 78.9%, and 81.3% for CAG, IM, Dys, and GC, respectively. SAPCD2 was overexpressed in precancerous lesions of the 121 cases of gastritis tissues examined (P≤0.001), with increasing expression correlated with the severity of precancerous tissue. Our IHC results show that SAPCD2 is overexpressed in GC tissues, however, these results should be confirmed with larger sample sizes in future studies.

In a previous study, we found that SAPCD2 is involved in gastric carcinogenesis by the virtue of suppressing the proliferation and tumorigenicity of BGC823 cells with the depletion of SAPCD2. The clinical, sample findings of the current study confirmed this role. Moreover, transfection of SAPCD2 into HepG2 and NIH3T3 cells (which have a low expression of SAPCD2) promoted their proliferation and tumorigenicity (19,20). Jung et al. reported that 9 mRNAs including that of SAPCD2 expressed at evident upregulated levels in colorectal carcinoma; a systematic analysis of human protein complexes involved in chromosome segregation showed that SAPCD2 may co-localize with centrosome protein CEP350 (21), which is required to stabilize microtubules of center body and poles during mitotic division (22,23). These results provide a hint that SAPCD2 may have a significant function in tumorigenesis. Therefore, the role and mechanism of

SAPCD2 in GC need to be studied; the generation of mAb such as the one described in this study is a critical step in investigating the SAPCD2 functions.

Clearly, our findings describe novel mAbs to SAPCD2 that also constitutes an advantageous and credible tool in fundamental research and clinical application of SAPCD2. These data provide the foundation for relevant studies of the SAPCD2 function in the development of GC and a large variety of human tumors.

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AUTHORS' CONTRIBUTIONS

W.S. conceived and designed the experiments, analyzed the results, and reviewed the manuscript. F. G. performed most of the experiments and drafted the manuscript. X. W. and W. Z. collected the patient samples and constructed the plasmid. J. L. and C. L. did the western blotting and immunoprecipitation. F. F. performed the ELISA experiment. Y. C. and W. H. did the cell culture experiment and PCR experiment. All authors read and approved the final version of the manuscript.

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AVAILABILITY OF DATA AND MATERIALS

All authors declare that the data generated or analyzed during this study are included in this published article and its supplementary information files.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments obeyed the protocols approved by the Animal Ethics Committee of the Pizhou Oriental Breeding Co. LTD. The study was carried out in accordance with ARRIVE guidelines and all methods were carried out in accordance with relevant guidelines and regulations. In addition, all patients provided informed consent to participate in the study, and the clinical study was approved by the Institutional Review Board of Huadu District People's Hospital.

Consent for Publication: Not applicable.

Conflict of Interest: None declared.

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