Original Article
Effects of SCAGP on the biology behavior of Gastric cancer MGC-803 cell

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Abstract: The current study demonstrated the effects of Sodium carboxyl-amino-glucan-polysaccharide (SCAGP) on the biology behavior of gastric carcinoma MGC-803 cells. In vivo, the mice inoculated intraperitoneally with gastric cancer MGC-803 cells were divided into control and treatment group (n = 20), which were intraperitoneally injected with saline (10 ml/kg body weight) and SCAGP (10 ml/kg body weight) daily for 10 days, respectively. After the mice were euthanized, the size and number of intraperitoneal cancer nodule were detected by laparotomy. In vitro studies, cell proliferation and survival assay were performed using the Cell Counting Kit-8 (CCK-8). Both of cell cycle distribution and cell apoptosis were identified by Flow Cytometry. Cell migration was detected by Transwell assay. The phosphorylation levels of several proteins involved in Akt and MAPK signal pathway were assessed by western blot analysis. Our data showed that in vivo SCAGP could markedly inhibit peritoneal metastasis of gastric cancer cells. In vitro, compared with the control group, SCAGP inhibited cell growth and proliferation, induced apoptosis and caused cell cycle arrest in the G0/G1 phase. The capacity of cellular migration was reduced significantly (P < 0.05). In addition, after treatment with SCAGP, the phosphorylation of AKT, extracellular signal-regulated kinase1/2 (ERK1/2) and c-Jun N-terminal kinases (JNK) were down-regulated, with the phosphorylation of p38 mitogen-activated protein kinase up-regulated in a dose-dependent manner. This study suggested that SCAGP may serve as a new kind of anti-tumor drug for gastric carcinoma treatment in the future.

Keywords: Sodium carboxyl-amino-glucan-polysaccharide (SCAGP), gastric carcinoma (GC), MGC-803 cells, peritoneal metastasis (PM), Akt, MAPK

Introduction
Gastric cancer (GC) ranks first in various malignancies and is the second most common cause of cancer-related death in the world [1]. Although GC has become less common recently, it remains difficult to cure, primarily because most patients present with advanced disease, with a five-year survival rate lower than 30% [2], and increased to just about 60% after the popular application of gastrectomy with D2/D3 lymphadenectomy [3-5]. Peritoneal metastasis (PM) of exfoliated GC cells is an important factor leading to poor postoperative prognosis [6-8]. Therefore, the prevention and treatment of PM is the key to decrease the local recurrence and prolong survival in cases of advanced GC. And it has been a hot topic to search for an effective way to prevent and treat PM.

Sodium carboxyl-amino-glucan-polysaccharide (SCAGP), generally named SURG•TAKE, is a kind of biological colloid with a pH of 5.5-7.5. SCAGP is colorless or light yellow, non-cytotoxic, non-irritating, non-allergenic and belongs to chitosan biological. SCAGP is most often used to promote tissue repair and maintain organ surface smooth by washing the surgical wounds [9]. And it is a new drug typically used for intraoperative peritoneal lavage in the study of PM in GC. Recently, during the treatment of the patients taken with D2 radical gastrectomy, increasing studies have proved that peritoneal lavage with SCAGP can remove intraperitoneal exfoliated GC cells effectively and improve the effect of surgery [9, 10]. The main component of SCAGP is allantion (C$_4$H$_6$O$_3$N$_4$), which can be hydrolyzed into urea and mandelic acid, then the former is discharged with uric acid and the
latter is transformed into acetoacetate [10]. Acetoacetate is a potent antioxidant, which can prevent the oxidation of tissue type plasminogen activator (t-pA) and contribute to keep t-pA to the critical value. When tissue is traumatized in clinical medicine, including surgical injury, t-pA will promote the repair of tissue, restore the serosal integrity, keep the serosal integrity and decrease the likelihood of PM in GC cells, unless it reaches a certain critical value [11, 12]. SCAGP plays a positive role in t-pA, and in this way, SCAGP promotes the repair of serosal thereby reduce the PM of GC cells [13]. Here, we provide direct evidences that SCAGP can inhibit PM of GC cells by injection experiment, suppress the biology behavior of GC MGC-803 cells related to implantation metastasis, and reduce the phosphorylation of Akt, extracellular signal-regulated kinases (ERKs) and c-Jun N-terminal kinases (JNK), but increase the phosphorylation level of p38 mitogen-activated protein kinase.

Materials and methods

In vivo assay

Forty healthy mice between 8 and 10 weeks old were obtained from Shanghai Experimental Animal Center, Chinese Academy of Science (Shanghai, China) and were housed in a room under controlled temperature (23 ± 1°C) with free access to water. Mice were fed standard chow for the first week to allow them to adjust to the new environment. Subsequently, all of the mice were injected intraperitoneally with GC MGC-803 cells. After this treatment, mice were divided into two groups randomly and treated in the following way daily for 10 days: one group of mice was injected intraperitoneally with SCAGP (10 ml/kg body weight) as our study group and the other group with saline (10 ml/kg body weight) as a control. Observed the physiological conditions of the mice every other day until all mice in one group died, then, all of the mice were euthanized. The size and number of the carcinoma nodule were detected through laparotomy. All of the above treatments were conducted under institutional guidelines for the humane treatment of laboratory animals.

Cell culture

The human GC cell line MGC-803 was obtained from Shanghai Institute of Cellular Biology (Shanghai, China), and cultured in RPMI-1640 medium (Hyclone Company, USA). All medium were supplemented with 10% heat-inactivated FBS (Gibco BRL, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. And the cells were cultured in an incubator (37°C, 100% humidity and 5% CO2, Thermo Fisher Scientific Inc., Waltham, MA, USA).

Cell growth and proliferation assay

Cell Counting Kit (CCK)-8 kit (Tongren, Shanghai, China) was used to assess the effects of SCAGP (Pi Er Fu Biological Technologies Co., Shan Xi) on cell viability. In brief, GC MGC-803 cells in logarithmic growth phase were collected and divided into six groups. Each group was treated with one cell culture broth for 60 mins, in which the corresponding concentrations of SCAGP were 0%, 10%, 20%, 30%, 40% and 50% (v/v), respectively. Then, the treated MGC-803 cells were plated at 3×10³ cells/well in 96-well plates with each group contained five parallel wells and incubated for 24 h. Subsequently, as a control, CCK-8 reagent was added to different concentrations of each well directly. Other culture plates were further incubated for 12 h, 24 h, 48 h and 72 h, respectively, and each of the time above was regarded as one treated group. Then, CCK-8 reagent was added to each treated group at 1 hour before the endpoint of incubation and the cell viability was evaluated. The optical density (OD) at 450 nm was determined for the supernatant of each well by a microplate reader. All experiments were performed at least three times.

Flow cytometry (FCM) detection

Cell cycles were measured using propidium iodide (PI) and flow cytometry. In the treatment group, MGC-803 cells were incubated in the cell culture solutions, containing 50% SCAGP (v/v) for 60 mins, with no SCAGP as a control. And then MGC-803 cells were seeded in 6-well plates at a density of 3×10⁵ cells/well. After being incubated for 24 h, the study group was continued to incubate for 24 h, 48 h and 72 h, with control group not processed. Then, cells were washed with PBS, trypsinized and centrifuged at 1000 x g, 4°C for 5 mins. The obtained pellets were suspended with 300 μL PBS containing 10% FBS, and fixed in 700 μL cold ethanol at -20°C for 24 h. Then, cells were washed...
twice with PBS and incubated in PBS containing RNase A (1 mg/mL) for 10 mins at 37°C. The samples were stained with PI (1 mg/mL) for 10 mins shielded from light at the room temperature. At last, the cells were analyzed by flow cytometry (FACS Calibur, BD Biosciences, Franklin Lakes, NJ, USA) and the data were analyzed using Cell Quest software (BD Biosciences).

On the other hand, apoptotic cells in different group (treatment with 50% SCAGP & time-based grouping) were identified by Flow Cytometry and the AnnexinV/fluorescein isothiocyanate (FITC) kit (BD Biosciences). Briefly, MGC-803 cells were collected, resuspended in 200 μL of binding buffer containing 5 μL of Annexin V-FITC, stained at (20-25)°C in the dark for 10 mins, centrifuged at the room temperature 1000 ×g for 5 mins, resuspended in 200 μL of binding buffer again, then added 10 μL of PI, mixed gently and stained in an ice bath dark place. After the above assays, flow cytometric analyses were performed.

**Cell migration assay**

MGC-803 cells in different group (treatment with 50% SCAGP & time-based grouping) were starved with serum-free RPMI-1640 medium (Hyclone) for 24 h before the following assays. Cells were digested for 5 mins by 0.25% trypsin (Gibco, Shanghai, China) and resuspended in RPMI-1640 medium (Hyclone) containing 0.1% BSA. After counting, cells were diluted to 5×10^5 cells/mL, seeded in a 24-plate transwell room and then continued to incubate for 24 h, 48 h and 72 h. Then, cells were fixed for 15 mins using 1 mL/well 4% paraformaldehyde (JRDUN biotech, Shanghai, China), stained by Giemsa (JRDUN) for 30 mins and washed 3 times by 1× PBS. Finally, wipe the transwell room carefully without migrating cells using a cotton swab, placed it under 200× microscope and counted the number of cells in random fields of 5-10.

**Western blot**

After treatment with SCAGP at the desired concentrations and time, cultured cells were harvest and washed twice with PBS. Cell lysates were prepared in a buffer containing immunoprecipitation assay buffer (RIPA, Beyotime, Shanghai, China), as well as 0.01% protease and phosphatase inhibitor (Sigma, Shanghai, China) and then were incubated at 4°C for 10 mins, heated at 95°C for 10 mins, centrifuged at the room temperature 12000 ×g for 10 mins, finally the protein in supernatant was obtained. After the protein was quantitation using BCA kit (PICPI23223, thermo), equal amounts of proteins (20-30 μg) were separated by SDS-PAGE and bolted onto nitrocellulose membranes by the semi-dry blotting method using a three buffer system. The membrane was blocked with 5% BSA in TBST and incubated a 1:1000 dilution of primary antibody (anti-p-AKT, anti-p-ERK, anti-p-JNK and anti-p-p38) (CST, USA) overnight at 4°C. Then the membrane was washed with TBST three times and incubated for 1 hour at 37°C temperature with secondary antibody (with HRP tag, 1:1000). The signals were revealed by enhanced chemiluminescence ECL (Millipore, USA) after another around of TBST wash. Intensities were measured using Image J (NIH, USA). GAPDH antibody (1:1500) (CST, USA) was used to confirm equal sample loading. The experiment was repeated three times independently.

**Statistical analysis**

Experiments were performed with values expressed as mean ± SD. Statistical analysis was determined using Graphpad Prism 6.0 software. Comparisons between multiple groups were analyzed using Two-Way ANOVA, with column analyses using T tests (and non-parametric tests). In all statistical comparisons, P < 0.05 was considered as significant difference. All experiments were independent and performed at least three times.

**Results**

SCAGP markedly inhibits peritoneal metastasis (PM) of gastric cancer (GC) MGC-803 cells

In vivo assay, all the mice carrying MGC-803 GC cells were divided into two groups, which were treated with SCAGP and saline, respectively. The size and number of liver nodules, mesenteric nodules and pelvic cavity wall nodules were observed as the metrics of PM in all the mice carrying MGC-803 GC cells. Our results showed that the saline treatment group gener-
SCPGA affect the biology behavior of gastric MGC-803 cells

SCAGP markedly inhibit peritoneal metastasis of gastric cancer cells. The saline treatment mice were as a control group (A-C). Liver nodules (A). Mesenteric nodules (B). Pelvic wall nodules (C). The SCAGP treatment group mice, the liver, mesenteric and pelvic presented nearly normal (D-F). Liver without nodules (D). Mesenteric without nodules (E). Pelvic wall with one nodule (F).

SCAGP inhibits cell growth and proliferation in gcmgc-803 cells

To figure out the effect of SCAGP on MGC-803 cells growth and proliferation, the survival rates

Figure 2. Cell Survival was Assessed by (CCK)-8 Assay. The growth of MGC-803 cells was markedly obstructed by SCAGP in a time and dose-dependent manner ($P < 0.01$) (A). The mean cell proliferation rates of MGC-803 cells which were incubated with different concentrations of SCAGP for 72 h were significantly lower than that of the control cells which were unprocessed (B). $n = 8$, mean ± SD. ***$P < 0.001$ vs. control group (SCAGP, 0%).

ated a lot big nodules in liver, mesenteric and pelvic cavity wall, while the SCAGP treatment group mice were presented nearly no nodules (Figure 1), suggesting that SCAGP inhibited PM of GC MGC-803 cells.

**SCAGP inhibits cell growth and proliferation in gcmgc-803 cells**

To figure out the effect of SCAGP on MGC-803 cells growth and proliferation, the survival rates
SCPGA affect the biology behavior of gastric MGC-803 cells

To substantiate cell apoptosis induced by SCAGP treatment under various time, annexin-V FITC/PI double staining and flow cytometry analysis were performed. The number of apoptotic cells was as shown in the lower right quadrant of MGC-803 cells in vitro was determined. The results showed that the growth of MGC-803 cells was markedly obstructed by SCAGP in a time and dose-dependent manner (n = 3, P < 0.01). Compared with the control group, there was a significant statistical difference in the group with different time and concentrations and this effect has an enlarge mechanism (Figure 2A). The mean cell proliferation rates of MGC-803 cells which were incubated with different concentrations of SCAGP for 72 h were significantly lower than that of the control cells which were unprocessed (n = 3, P < 0.01) (Figure 2B). The result indicated that SCAGP could inhibit cell growth and proliferation in GC MGC-803 cells.

Effects of SCAGP on cell cycle control in GC MGC-803 cells

In order to determine whether the inhibitory effect of SCAGP on MGC-803 cell proliferation was mediated by cell cycle, flow cytometry was used. The results revealed that compared with the control group, each group cells in G0/G1 phase were significantly increased with time, while cells in S phase were impaired by 11.77%, 15.41% and 18.58%, respectively; along with G2/M phase cells were impaired by 4.85%, 4.68% and 40.22%, respectively (n = 3, P < 0.01). SCAGP induced cell cycle arrest at G0/G1 phase, suggesting that SCAGP suppressed cell proliferation by controlling the G0/G1 checkpoint and inducing a specific block in cell cycle progression (Figure 3).

SCAGP induces cellular apoptosis

Figure 3. Effects of SCAGP on the cell cycle of MGC-803 cells. The cell cycle was analyzed by PI stain assay and flow cytometry after MGC-803 cells were treated with SCAGP at the dose of 50% for 24 h, 48 h and 72 h (A). The percentage of G0-G1 phase cells in MGC-803 cells were increased while the percentages of S and G2-M phase cells were decreased in a time-dependent manner (B). n = 8, mean ± SD. *P < 0.05, **P < 0.01 vs control group (Time, 0 h).
SCPGA affect the biology behavior of gastric MGC-803 cells

Figure 4. Effects of SCAGP on apoptosis of MGC-803 cells. Cell apoptosis was substantiated by Annexin-V/PI double stain assay and flow cytometry analysis (A). Treatment of SCAGP at the dose of 50% for 24 h, 48 h and 72 h time-dependently increased the apoptotic population of MGC-803 cells (B). n = 8, mean ± SD. ***P < 0.001 vs. control group (Time, 0 h).

Figure 5. Effects of SCAGP on migration of MGC-803 cells. Migration ability of MGC-803 cells was identified by Transwell assay at various time (A). The migration number of MGC-803 cells was markedly declined by SCAGP in a time-dependent manner (B). n = 8, mean ± SD. **P < 0.01, ***P < 0.001 vs control group (Time, 0 h).

As shown in Figure 4, the apoptosis rate of MGC 803 cells was 2.27% ± 0.004 (n = 3, P < 0.01) in control group. At 24 h, 48 h and 72 h after SCAGP treatment, the number of apoptotic cells were significantly increased, and their apoptosis rates were 11.9% ± 0.002, 21.03% ± 0.009 and 28.60% ± 0.021, respec-
SCPGA affect the biology behavior of gastric MGC-803 cells


tively (n = 3, P < 0.01). The results suggested that SCAGP induced GC MGC-803 cells apoptosis and the effects were enhanced along with the increased time.

**SCAGP inhibits migration of MGC-803 cells**

In order to explore if SCAGP has an effect on migration of MGC-803 cells, the transwell migration test was performed and results were showed in Figure 5. Compared with the control group, treatment with SCAGP obviously suppressed the migration ability of cells in a time-dependent manner, and the number of cell migration in the control group was 107 ± 5, while at 24 h, 48 h and 72 h after SCAGP treatment, the number of cell migration were 79 ± 6, 44 ± 5 and 35 ± 3, respectively (n = 3, P < 0.01).

**Effects of SCAGP on proteins expression in MGC-803 cells**

To investigate the molecular mechanism of MGC-803 cells biology behavior induced by SCAGP, the phosphorylation levels of several kinases were detected by western blot. As shown in Figure 6, p-AKT, p-ERK1/2 and p-JNK were down-regulated, and p-p38 was up-regulated, all of which were in a dose-dependent manner. Treatment with high concentration of SCAGP (≥ 30%) brought more obvious changes in the phosphorylation levels than low concentration of SCAGP (< 30%). These data indicated that SCAGP impaired the proliferation and migration, induced apoptosis, as well as caused cell cycle arrest in G0/G1 phase of MGC-803 cells via the Akt and MAPK signaling pathways.

**Discussion**

Metastasis is the basic biological characteristics of malignant tumors, and it is also the main reason for cancer patients’ death [14, 15]. Recently, “blocking therapy”, such as radiation treatment and chemotherapy, has been considered as the main method to inhibit the metastasis of advanced GC and thereby improve the survival rate, but the effects are not yet very satisfactory [16]. Nearly since SCAGP appeared, it has been implicated in PM of malignant tumors, especially GC. However, the mechanism about how SCAGP influences tumor is still far from being fully understood [9, 10].

Our in vivo and in vitro study demonstrated that SCAGP markedly inhibited PM and cell viability of MGC-803 cells, induced cell apoptosis and cell cycle arrest in G0/G1 phase, as well as reduced the capability of migration. All of the functions of SCAGP on MGC-803 cells may be related with the components of SCAGP. Allantion (C_{4}H_{6}O_{3}N_{4}), as the main component of SCAGP, can be hydrolyzed into urea and man-
SCPGA affect the biology behavior of gastric MGC-803 cells

delic acid, then the former is discharged with uric acid and the latter is transformed into acetacetate [10]. Acetacetate is a metabolic inhibitor of cancer growth [17]. Moreover, in the over-express uncoupling protein2 cancer cell lines, acetacetate reduces growth and ATP concentration of cancer cells [18]. On the other hand, SCAGP is a kind of carboxyl-amino-glucan-polysaccharide polymer derivative. The polymer derivative of carboxyl-amino-glucan-polysaccharide can bind with tumor cells, viral and bacterial surface, thereby, the recombinant antigen is formed, which is easier to be recognized than just the tumor cells, and contributes to the immune system being activated [19, 21]. Moreover, SCAGP, as a polymer derivative of carboxyl-amino-glucan-polysaccharide, may combine with the tumor cell surface substance, lead to changes in the function of cell membrane, and increased cell permeability, at last, cell rupture and die [20, 22]. Although the mechanism of their antitumor action is still not completely clear, many polysaccharide-protein complexes are suggested to enhance cell-mediated immune responses in vivo and in vitro and act as biological response modifiers [22]. To sum up, the effects of SCAGP on the biology behavior of GC MGC-803 cells and its related mechanism are reasonable and can be predicted.

In addition, phosphorylation of Akt and MAPK signaling were found adjusted by SCAGP in a dose-dependent manner. Akt, involved in cellular survival pathways, plays a key role in multiple cellular processes such as regulating glucose metabolism, suppressing apoptosis, promoting cell proliferation and cell migration [23]. MAPK, a chain of proteins in the cell that communicates the signal from a receptor on the cell surface to the DNA in the cell nucleus, can be divided into three subfamilies: ERK, p38, JNK [24]. Among them, ERK1/2 have a negative effect on apoptosis and cell proliferation [23, 25]; p38 involves in cell survival, differentiation and plays a positive role in apoptosis in tumor cells [26]; JNK is also associated with apoptosis but the research about its effect on apoptosis is controversial. There are reports that in gastrointestinal cancers, suppressing the activation of JNK using its inhibitor SP-600125 can induce apoptosis of cancer cells [27]. JNK1 disruption increased hepatocyte death and susceptibility to HCC formation [28]. Still, there are researches that preventing the activation of JNK can prolong the cell survival rates [29]. In this study, the phosphorylation levels of AKT, ERK1/2 and JNK were down-regulated, and p-p38 was up-regulated, all of which were in a dose-dependent manner. The result indicated that the effect of SCAGP on biology behavior of GC MGC-803 cell may work via Akt and MAPK signaling pathway. Treatment with high concentration of SCAGP (≥ 30%) brings more obvious changes in the phosphorylation levels than low concentration of SCAGP (< 30%). The data suggested that if SCAGP was recognized as a new drug for cancer therapy in the future, 30% would be a crucial concentration of SCAGP in further research and offer a reference concentration for therapeutic drug monitoring (TDM). On the other hand, the phosphorylation of Akt and MAPK may activate its downstream factor S6K1 and 4EBP-1, resulting in increased apoptosis [30], which is consist with the results of our cell cycle experiments exactly.

Conclusion

The results from our study demonstrate that SCAGP can inhibit PM of GC cells and suppress the biology behavior of GC cell related to implantation metastasis, which provide a prominent choice for irrigation of peritoneal cavity for the radical operation of advanced gastric carcinoma. SCAGP may serve as a new drug for gastric carcinoma treatment.

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Disclosure of conflict of interest

None.

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SCPGA affect the biology behavior of gastric MGC-803 cells

References


SCPGA affect the biology behavior of gastric MGC-803 cells


[27] Cano E and Mahadevan LC. Parallel signal processing among mammalian MAPKs. TIBS 2007; 20: 117.

