

Antiproliferative effect of octreotide on gastric cancer cells mediated by inhibition of Akt/PKB and telomerase

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Abstract

AIM: To investigate the antiproliferative effect of octreotide, a long-acting analogue of somatostatin, on gastric cancer cell line SGC7901 and its possible molecular mechanisms.

METHODS: Gastric cancer cell line SGC7901 employed in the study was treated with 0.008, 0.04, 0.2, 1, 5 and 25 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide respectively for 24 h to evaluate the antiproliferative effect of somatostatin analog on the tumor cells by MTT assay method. To elucidate the underlying mechanism, the cells were exposed to 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide for 0, 12, 24 and 48 h, when their Akt/PKB and telomerase activities were respectively determined using PCR-ELISA and nonradioactive protein kinase assay protocols. The same experimental procedures were also performed in the control cells that were treated with corresponding vehicles instead of somatostatin analog.

RESULTS: After exposed to octreotide for 24 h at the concentrations of more than 1 $\mu\text{g}\cdot\text{ml}^{-1}$, SGC7901 cells exhibited a dose-dependent inhibition of growth with the inhibiting rate to be as high as 34.66 % when 25 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide was applied. The Akt/PKB and telomerase activity of SGC7901 cells was significantly inhibited when the cells were exposed to 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts ($P<0.01$), both of which exhibited in a time-dependent manner.

CONCLUSION: The antiproliferative effect of octreotide on SGC7901 cells might be mediated by the inhibition of Akt/PKB and telomerase.

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INTRODUCTION

Gastric cancer continues to be one of the most common malignancies in human worldwide^[1-4]. In China, its average rate of annual mortality is estimated to be as high as 16 per 100 000 population that accounts for the leading cause of death among malignant tumors^[5-7]. Due to the limited efficacy and

considerable toxicity of conventional chemotherapy, some novel cytotoxic and noncytotoxic agents such as certain dietary substances, natural hormones and synthetic compounds have been proposed to stop or reverse the process of carcinogenesis^[8], in which increasing attention has been paid to somatostatin and its analogs that have been reported to exert antineoplastic effects in a wide range of tumor types such as carcinoid, osteosarcoma, leukemia, and cancers of thyroid, breast, lung, liver, pancreas, colon, as well as gastric carcinomas^[9-19]. However, detailed mechanisms underlying the antineoplastic actions of somatostatin and its analogs that remain to be clarified have limited the therapeutic efficacy of these agents in the treatment of clinical malignancies^[20, 21].

Recent advances in molecular biological science have revealed that some molecules such as telomerase and protein kinase B or Akt (PKB/Akt) are involved in the process of carcinogenesis. Telomerase is an enzyme that replaces repetitive (TTAGGG)_n sequences on the ends of chromosomes that would otherwise be lost during successive cell divisions. Its activation is closely linked to the attainment of cellular immortality and considered to be an important step in tumorigenesis^[22-26]. Another molecule PKB/Akt, a serine/threonine kinase, has also recently been a focus of intense research. It appears that Akt/PKB lies in the crossroads of multiple cellular signaling pathways and acts as a transducer of many functions initiated by growth factor receptors. It is particularly important that Akt/PKB can mediate cell survival and serve as a critical factor in the genesis of malignancies^[27-30]. Thus, an investigation to clarify how the antiproliferative effects on these couple of molecules might be helpful in elucidating antineoplastic mechanisms of somatostatin analogs, which is no doubt of both theoretical and practical importance.

MATERIALS AND METHODS

Materials

Human gastric cancer cell line SGC7901 was obtained from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Somatostatin analogue octreotide was provided by Sandoz Corp, RPMI1640 and fetal bovine serum (FBS) were from Gibco. 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), telomerase TRAP PCR-ELISA kit and Akt/PKB kinase assay kit were purchased from Sigma, Roche Molecular Biochemicals and CST respectively. Microtiter plate reader was made by Digiscan, Austria ASYS Hitech.

Cell culture

SGC7901 cells were maintained in flasks with RPMI-1640 supplemented with 10 % heat-inactivated FBS, 100 U·ml⁻¹ penicillin, 100 U·ml⁻¹ streptomycin and 20 mM sodium bicarbonate at 37 °C in a humidified atmosphere containing 5 % of CO₂. The culture medium change was performed every 3 days.

MTT assay

A MTT assay was conducted to determine the cell proliferation. In brief, SGC-7901 cells were seeded at 5×10³·wells⁻¹ in a 96-

well plate and incubated in 200 μl of culture medium overnight. Then the cells were made quiescent by serum deprivation and treated with 0.008, 0.04, 0.2, 1, 5, 25 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide respectively as the different observatory groups. The cells treated with equal amount of normal saline instead of octreotide served as the control. After cultivated for 24 h, 20 μl of stock MTT solution (2.5 $\text{mg}\cdot\text{ml}^{-1}$) was added to cells for each well which were further incubated at 37 $^{\circ}\text{C}$ for 4 hours. The culture medium was carefully removed followed by adding of 150 μl of DMSO to each well and vibrating culture well for 10 min. The absorbance of samples was measured at a wavelength of 490 nm with a microtiter plate reader. The negative control was conducted using only cell-free culture medium and each assay was performed in triplicate. The inhibiting rate of cell growth was expressed as $(A-B)/A\times 100\%$, where A is the absorbance value from the controls and B is that from the experimental cells.

Immunoprecipitation kinase assay

Akt/PKB activity was examined by the immunoprecipitation kinase assay. Briefly, 5×10^5 of SGC7901 cells were seeded in a 25- cm^2 flask and cultured for 3 days. When being confluent at 70%, cells nominated as group A were incubated in serum-free RPMI-1640 for 24 h and then treated with 1 $\mu\text{g}\cdot\text{ml}^{-1}$ octreotide for further cultivation of 0-, 12-, 24- and 48-h respectively. Same procedures were also performed in group B cells that served as the control except being treated with equal volume of normal saline instead of octreotide. Confluent cells were washed three times in cold PBS, then lysed in buffer containing 20 $\text{mmol}\cdot\text{L}^{-1}$ Tris-HCl (pH 7.5), 150 $\text{mmol}\cdot\text{L}^{-1}$ NaCl, 1% Triton X-100, 1 $\text{mmol}\cdot\text{L}^{-1}$ EDTA, 1 $\text{mmol}\cdot\text{L}^{-1}$ EGTA, 2.5 $\text{mmol}\cdot\text{L}^{-1}$ sodium pyrophosphate, 1 $\text{mmol}\cdot\text{L}^{-1}$ β -glycerophosphate, 1 $\text{mmol}\cdot\text{L}^{-1}$ Na_2VO_4 , 1 $\mu\text{g}\cdot\text{ml}^{-1}$ leupeptin and 1 $\text{mmol}\cdot\text{L}^{-1}$ phenylmethyl-sulfonyl fluoride (PMSF) for 10 min at 4 $^{\circ}\text{C}$. Samples were microcentrifuged for 10 min and 200 μl of the cell lysate was incubated with 20 μl of resuspended immobilized Akt antibody slurry at 4 $^{\circ}\text{C}$ for 2 h to immunoprecipitate Akt/PKB, the latter was next used to phosphorylate a specific substrate, GSK-3 fusion protein (Ser21/9). That is, 1 μg of GSK-3 fusion protein was incubated with immunoprecipitated Akt/PKB in the presence of ATP for 30 min at 30 $^{\circ}\text{C}$ followed by adding 20 μl of 3 \times SDS sample buffer to terminate the reaction. Samples were then boiled for 5 min, separated by 12% SDS-PAGE and transferred onto nitrocellulose membrane. Nonspecific reactivity was blocked by 5% fat-free milk in TBST (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 1 h. The membrane was incubated with a 1:1 000 dilution of phospho-GSK-3 α/β antibody for 2 h, then with HRP-conjugated anti-rabbit (1:2 000) and anti-biotin antibody (1:1 000) for 1 h at room temperature. Finally, reactive protein was revealed by incubating with 10 ml of LumiGLO for 1 min and exposed to x-ray film for 1-2 min at room temperature.

Determination of telomerase activity

The telomerase activity was determined by using TRAP-ELISA. 5×10^5 of SGC7901 cells were seeded in a 25- cm^2 flask and cultured for 3 days. When being confluent at 70%, cells nominated as group A were incubated in serum-free RPMI-1640 for 24 h and then treated with 1 $\mu\text{g}\cdot\text{ml}^{-1}$ octreotide for further cultivation of 0-, 12-, 24- and 48-h respectively. Same procedures were also performed in group B cells that served as the control except being treated with equal volume of DMSO instead of octreotide. Confluent cells were harvested and centrifuged at 3 000 g for 10 min at 4 $^{\circ}\text{C}$. After resuspended, 5×10^3 cells were lysed in an Eppendorf tube, whose extract was next used to amplify telomeric repeat. The telomerase activity was determined with PCR-ELISA according to the

manufacturer's protocol. Sample absorbance was measured with a microtiter plate reader at the wavelength of 450/690 nm within 30 min after addition of the stop reagent.

Statistical analysis

Experimental data were analyzed by ANOVA and Student's *t* test for multiple comparisons between groups. The data were finally expressed as mean \pm standard error of the mean. *P* value less than 0.05 was considered statistically significant.

RESULTS

Antiproliferative effect of octreotide on SGC7901 cells

When exposed to octreotide for 24 h at the concentration of more than 1 $\mu\text{g}\cdot\text{ml}^{-1}$, SGC7901 cells exhibited a dose-dependent inhibition of growth with the inhibiting rate to be as high as 34.66% when 25 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide was applied (Table 1).

Table 1 Antiproliferative effect of octreotide on SGC7901 cells

Octreotide ($\mu\text{g}/\text{ml}$)	OD ₄₉₀	IR (%)
Control	0.528 \pm 0.032	/
25	0.345 \pm 0.041	34.66 ^b
5	0.436 \pm 0.022	21.21 ^b
1	0.465 \pm 0.019	11.93 ^a
0.2	0.493 \pm 0.008	6.63
0.04	0.510 \pm 0.031	3.41
0.008	0.504 \pm 0.065	0.76

^a*P*<0.05, ^b*P*<0.01 vs control.

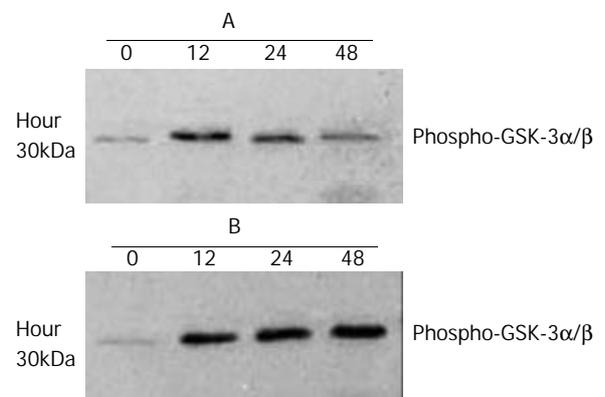


Figure 1 Inhibitory effects of octreotide on Akt/PKB activity in SGC7901 cells. The Akt/PKB activity in group A SGC7901 cells was significantly inhibited after the cells were exposed to 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts (Figure 1B), which manifested in a time-dependent manner as shown in Figure 1A.

Inhibition of Akt/PKB activity

The Akt/PKB activity in group A SGC7901 cells was significantly inhibited after the cells were exposed to 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts (Figure 1B), which exhibited in a time-dependent manner as shown in Figure 1A.

Inhibition of telomerase activity

Just like that of the Akt/PKB, the telomerase activity in group A SGC7901 cells was also significantly inhibited after the cells were exposed to 1 $\mu\text{g}\cdot\text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts (*P*<0.01), which exhibited in a time-dependent manner as shown in Figure 2A.

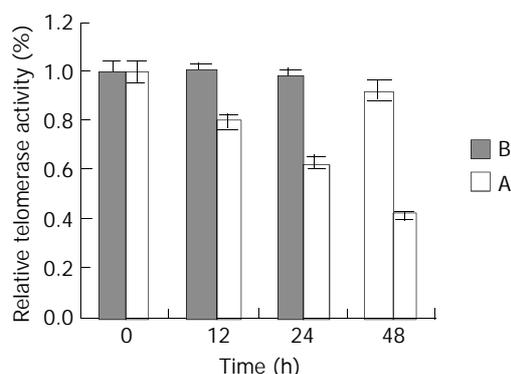


Figure 2 Inhibitory effects of octreotide on telomerase activity in SGC7901 cells. The telomerase activity in group A SGC7901 cells was significantly inhibited after the cells were exposed to $1 \mu\text{g} \cdot \text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts ($P < 0.01$), which exhibited in a time-dependent manner as shown in Figure 2A.

DISCUSSION

Over the past decade, impressive antineoplastic effects of somatostatin and its analogs have been reported in plenty of tumor models and cancer cell types^[9-19]. A more recent research has further provided considerable information regarding the mechanisms underlying the antiproliferative and apoptosis-inducing actions of these compounds. These included both "direct" mechanisms that were the sequellae of binding of somatostatin analogs to their corresponding receptors present on neoplastic cells and "indirect" mechanisms that might be the result of reduced or inhibited secretion of growth-promoting hormones and growth factors that stimulated the growth of various types of malignancies^[21,31-33]. Apart from these discoveries, some biological actions such as the inhibition of angiogenesis and the negative influence on the immune system were also considered to be the important factors contributing to the antineoplastic effects of somatostatin analogs^[21,34], which therefore suggested that the latter might be involved in an even wider mechanism.

In the present study, we observed the inhibitory effects of octreotide on gastric cancer SGC7901 cells, as well as on the activities of Akt/PKB and telomerase. As to our knowledge, it is one of the fewer reports in recent years that concerned about this subject. It revealed that after exposed to octreotide for 24 h at the concentration of more than $1 \mu\text{g} \cdot \text{ml}^{-1}$, SGC7901 cells exhibited a dose-dependent inhibition of growth with the inhibiting rate to be as high as 34.66 % when $25 \mu\text{g} \cdot \text{ml}^{-1}$ of octreotide was applied. Furthermore, in a previous study, we also noted that this inhibitory action could not be abrogated by supplementing 10 % FBS to the cultured cells, which only prolonged the time course of cell death (data to be published). It indicated the mitogenic action provided by serum growth factors in the culture medium could be overcome by octreotide. Akt/PKB and telomerase are the enzymes that have recently been considered to be closely related to carcinogenesis^[22-30]. Whether the activation of Akt/PKB was one of the critical factors in cell to determine either a survival or an apoptotic message was conducted on the signal passway^[35]. Amplification of genes encoding Akt/PKB isoforms has been found in several types of human cancers^[36]. In addition, Akt/PKB has been reported to promote tumor progression and invasiveness by enhancing angiogenesis^[28]. So far as the telomerase is concerned, its activity has been detected in over 90 % of human cancers^[37-41]. In gastric cancer, telomerase was activated very early in the process of this disease^[42], which suggested the activation of telomerase might be also a determining factor contributing to the tumorigenesis. The relationship between

Akt/PKB and telomerase has also been noted by Kang and his coworkers^[43] with the founding that Akt kinase enhanced human telomerase activity through phosphorylation of hTERT subunit as one of its substrate proteins in SK-MEL28 cells. In the present study, we found that both activities of Akt/PKB and telomerase in SGC7901 cells were significantly inhibited after the cells were exposed to $1 \mu\text{g} \cdot \text{ml}^{-1}$ of octreotide for 12, 24 and 48 h compared with that of their control counterparts. Based on these findings, it can be concluded that the antiproliferative effects of somatostatin analogs on SGC7901 cells might be mediated at least in part via a mechanism of inhibition of Akt/PKB and then telomerase. Our further study should aim at elucidating the components on the signal conduction passway in up and downstreams of these events.

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