

# Octreotide inhibits the proliferation of gastric cancer cells through P300-HAT activity and the interaction of ZAC and P300

LIPING WANG<sup>1</sup>, XIN HUANG<sup>1</sup>, YURONG CHAI<sup>1</sup>, LIYANG ZOU<sup>1</sup>, MATTHEW CHEDRAWE<sup>2</sup> and YI DING<sup>1</sup>

<sup>1</sup>Department of Histology and Embryology, College of Basic Medical Sciences, Zhengzhou University, Zhengzhou, Henan 450001, P.R. China; <sup>2</sup>Department of Psychology and Neuroscience, Faculty of Science, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada

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**Abstract.** Somatostatin (SST) exhibits a wide range of physiological functions, including the regulation of tumor cell growth. Octreotide (OCT) is a synthetic analogue of SST that can be used to slow gastrointestinal bleeding, inhibit the release of growth hormone and impede gastrointestinal tumor growth. The aim of the present study was to investigate the molecular mechanism of OCT underlying the inhibition of gastric cancer cell proliferation. Proteins of interest were detected using western blotting, and the zinc finger protein (ZAC)-P300 complex was quantified using co-immunoprecipitation. P300-histone acetyltransferase (P300-HAT) activity was determined spectrophotometrically. The results showed that OCT decreased the phosphorylation of Akt which caused the level of ZAC to increase. In turn, the interaction between ZAC and P300 increased the activity of P300-HAT; ultimately, the phosphorylation of serine 10 in histone H3 (pS10-H3) was decreased and the acetylation of lysine 14 in histone H3 (acK14-H3) was increased. These results suggest that OCT attenuates SGC-7901 cell proliferation by enhancing P300-HAT activity through the interaction of ZAC and P300, causing a reduction in pS10-H3 and an increase in acK14-H3. These findings provide insight for future research on OCT and further demonstrate the potential of OCT to be used as a therapeutic agent for gastric cancer.

## Introduction

Numerous post-translational modifications (PTMs) exist to further specify protein function; some examples include histone phosphorylation, acetylation, methylation and

ubiquitination (1). Histone PTMs are the points of convergence in signaling pathways and these nodal events are crucial for gene expression (2). Different histone PTMs have three major distinct outcomes. Firstly, cooperative interactions: two or more signals collaborate to promote protein stabilization or recruitment. Secondly, mutually exclusive PTMs: PTMs can be mutually exclusive, however, these different PTMs cannot occur simultaneously. Thirdly, antagonistic PTMs: a PTM that is attached to one amino acid can, for example, antagonize the ability of an adjacent modified residue to recruit a binding partner (3). Histone H3 is a substrate of signal kinase and its phosphorylation specificity involves cell cycle progression and gene expression regulation (4). An increasing number of histone modifying complexes are found to have more than one distinct enzymatic activity. These enzymes can act in concert to determine the functional status of chromatin by coordinating multiple histone modifications. It is now well established that there is intense crosstalk between histone modifications to drive distinct downstream functions as well. Cross-regulation can occur in different ways; one modification can promote/block the addition of another modification, while another modification can stimulate/block the removal of another (5).

Akt plays an important role in the growth factor signaling pathway and can regulate cell growth, transcription and nutrition metabolism (6). If the Akt signaling pathway is inhibited, it may therefore lead to tumor cell death and growth inhibition (7). Zinc finger proteins (ZACs) are transcription factors that have transactivation and DNA-binding functions which can induce apoptosis and cell cycle arrest (8). P300 is a transcription cofactor with histone acetyltransferase (HAT) activity which is capable of acetylating all four core histones as well as over 70 other proteins. Its histone HAT domain is the core domain by which it acetylates substrate proteins and promotes transcription (9-11). ZAC also acts as a transcriptional cofactor that works with HATs and histone deacetylases to regulate gene expression. Previous research has shown that the ZAC gene exhibits hypermethylation in gastric adenocarcinomas (12).

When somatostatin (SST) binds to the SST receptor (SSTR) it can regulate cell proliferation in normal tissues and tumors. The SSTR is a G protein-coupled receptor and Akt is one of the downstream molecules in the SSTR pathway (13,14). In

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*Correspondence to:* Yi Ding or Yurong Chai, Department of Histology and Embryology, College of Basic Medical Sciences, Zhengzhou University, 100 Kexue Road, Zhengzhou, Henan 450001, P.R. China

E-mail: dingyi@zzu.edu.cn

E-mail: yrchai@zzu.edu.cn

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gastric carcinomas, the expression of SST and ZAC are both downregulated implying that a positive correlation between SST and ZAC expression exists (15). Octreotide (OCT), an SST analogue, is able to upregulate the expression of the ZAC gene and inhibit the proliferation of gastric cancer cells (16). The inhibitory effect of OCT on the proliferation of gastric cancer cells was significantly reduced after siRNA knockdown of the ZAC gene. This suggests that ZAC plays an important role in OCT signaling to inhibit the proliferation of gastric cancer cells (16).

Simultaneous induction of histone phosphorylation and acetylation is crucial for the activation of specific mammalian genes. For instance, the phosphorylation and acetylation of histone H3 can influence the regulation of cell proliferation gene expression (17). Histone H3 serine residue phosphorylation is a highly dynamic process and the phosphorylation of serine 10 on histone H3 (pS10-H3) plays a significant role in the NF- $\kappa$ B pathway of gene transactivation (18). S10-H3 phosphorylation is closely related to the acetylation of lysine 14 on histone H3 (acK14-H3) and acK9-H3 (19); in fact, pS10-H3 has been shown to stimulate acK14-H3 via GCN5, a prototypical HAT that has a 10-fold preference for pS10-H3 over non-phosphorylated H3 as a substrate *in vitro* (20-22).

Although effort has been made to elucidate how OCT works, the exact mechanism is yet to be determined. The purpose of the present study was to determine the inhibitory mechanism of OCT in gastric cancer cells. More specifically, OCT was proposed to increase the activity of P300-histone acetyltransferase (P300-HAT) in gastric cancer cells by downregulating p-Akt and upregulating ZAC. We anticipated that acK14-H3 and pS10-H3 are target sites of P300; therefore, OCT may be able to regulate pS10-H3 and acK14-H3 to inhibit gastric cancer cell proliferation.

## Materials and methods

**Cell culture.** A human gastric carcinoma cell line (SGC-7901) was provided by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and routinely cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA). The medium was supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin (both from Sigma-Aldrich, St. Louis, MO, USA) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. OCT, which was used to treat cells, was obtained from Beijing Dingguo Co. (Beijing, China) formulated as OCT acetate injection.

**Cell proliferation assay.** Cell proliferation was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability assay. Briefly, cells (5 $\times$ 10<sup>3</sup> cells/well) were plated in 96-well plates. The cells were treated with different concentrations of OCT (0, 1, 10, 100 and 1,000 nmol/l) for 24 h. At the end of each treatment, 10  $\mu$ l of MTT stock solution (5 mg/ml) was then added to each well, and the cells were incubated for an additional 4 h. The blue formazan salts produced from the cells were dissolved by adding 100  $\mu$ l of dimethyl sulfoxide (DMSO) and the absorbance of the blue solution at 490 nm was measured using a Microplate Reader Model 550 (Bio-Rad, Hercules, CA, USA). The cytotoxicity of OCT was determined by plotting the inhibitory

efficiency: IE% = [1 - (optical density of sample/optical density of control)]  $\times$  100%.

**Western blot analysis.** The SGC-7901 cells that underwent different treatments (10 nmol/l of OCT treatment for 12, 24 and 48 h, or no OCT) were collected after various time periods. Cells were then washed with cold phosphate-buffered saline (PBS) and lysed in ice-cold lysis buffer (10 mmol/l Tris-HCl, pH 8.0, 20% SDS, 1 mmol/l EDTA, 5 mmol/l DTT, 10 mmol/l PMSF) for 30 min at 4°C. Cell lysates were centrifuged at 13,400  $\times$  g for 10 min at 4°C and protein concentrations in the supernatants were determined using a bicinchoninic acid (BCA) protein assay (BCA assay kit; Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions. A total of 100 mg of protein was separated by 15% SDS-polyacrylamide gel electrophoresis (PAGE). After electrophoresis, gels were transferred to nitrocellulose/polyvinylidene fluoride (PVDF) membranes by electroblotting. The membranes were blocked with 5% non-fat dry milk in TBST (20 mmol/l Tris-HCl, pH 7.6, 137 mmol/l NaCl, 0.05% Tween-20) for 2 h at room temperature and incubated with primary antibodies (1:500) against Akt, p-Akt, ZAC, P300, pS10-H3 and acK14-H3 (Santa Cruz Biotechnology, Inc., Santa Cruz, USA), and total  $\beta$ -actin overnight at 4°C. After being washed three times for 20 min in TBST, the membranes were incubated for 1.5 h at room temperature with an appropriately diluted horseradish peroxidase-labeled secondary antibody (1:2,000; Zhongshan Jinqiao Beijing Biotechnology Co., Ltd., Beijing, China) in blotting buffer. The membranes underwent three 30-min washes in TBST with gentle shaking. The Akt, p-Akt, ZAC, P300, pS10-H3, acK14-H3 and total  $\beta$ -actin protein bands were visualized using the enhanced chemiluminescence (ECL) method. All proteins of interest were run alongside a housekeeping gene ( $\beta$ -actin, 1:1,000). The relative level of protein in the SGC-7901 cells was expressed as the gray value/ $\beta$ -tubulin.

**Spectrophotometric activity.** SGC-7901 cells received either 10 nmol/l of OCT treatment for 12, 24 and 48 h (experimental group), or no OCT treatment (control group). The nuclear proteins from all cells were extracted using the Nc-cell nucleus/plasma extraction kit (Beijing Dingguo Co.). The sample total activity and sample non-specific activity assays were performed using the kit following the manufacturer's instructions. The sample total activity and sample non-specific activity were evaluated via a UV-9000 spectrophotometer (Shanghai Yuanxi Instrument Co., Ltd., Shanghai, China).

The sample activity was calculated with the following equation:

$$\frac{[(SR - BR) \times 0.1 (SC: ml) SDM]}{[0.01 (SV: ml) \times 13.6 (MAC \times 15 (min))]} = \frac{\text{unit/ml}}{SPC (mg/ml)} = \text{unit/mg}$$

Here, SR signifies the sample reading, BR the background reading, SC the system capacity, SDM the sample dilution multiple, SV the sample volume, MAC the molar absorption coefficient and SPC the sample protein concentration. Unit = mMol Con A/min.

Sample specific activity = sample total activity - sample non-specific activity

**Co-immunoprecipitation assays.** Total proteins were extracted using the Nc-cell nucleus/plasma extraction kit obtained from Beijing Dingguo Co., and protein concentrations were determined using a BCA assay. The supernatant was then collected and incubated with an immunoprecipitating antibody [2 g (100  $\mu$ l) P300 antibody; Santa Cruz Biotechnology, Inc.] overnight at 4°C on a rotating wheel. Then, 40  $\mu$ l protein A agarose (Beijing Dingguo Co.) was added and incubation was carried out at 4°C overnight on a rotating wheel. Samples were then washed five times with lysis buffer and the final pellets were resuspended in 25  $\mu$ l of loading buffer (100 mmol/l Tris-HCl, pH 6.8, 200 mmol/l dithiothreitol, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 8% urea). Protein complexes were examined by western blotting with anti-ZAC (1:800; Santa Cruz Biotechnology, Inc.) to detect P300-ZAC interactions. Semi-quantitative analysis was carried out using ImageJ software and the band density indicated the relative amount of the P300-ZAC complex.

**Immunocytochemistry.** Immunocytochemistry was performed on slides with SGC-7901 cells after treatment with 10 nmol/l OCT for 12, 24 and 48 h and on those without OCT treatment. The slides were rinsed three times with 0.01 M PBS, fixed with 4% paraformaldehyde, incubated with 0.3% Triton X-100 in PBS for 1 h at room temperature, and placed in 1% H<sub>2</sub>O<sub>2</sub> in PBS for 30 min to quench endogenous peroxidases. The slides were then blocked with 5% bovine serum albumin (BSA) and incubated with primary polyclonal antibody [anti-pS10-H3 (1:200); anti-acK14-H3 (1:100); Santa Cruz Biotechnology, Inc.], and after washes with PBS a secondary antibody [biotinylated goat anti-rabbit (1:500)] was added. Next, the slides were incubated with a streptavidin-biotin complex (SABC) and processed with 3,3'-diaminobenzidine solution. A 0.1% methyl green staining solution was used to stain the cell nuclei.

All cells were scored based on immunoreactivity (IR) under a microscope. Cells with no IR were considered negative (0 points); cells with light brown granules were considered weakly positive (1 point); cells that had brown granules were considered positive (2 points).

**Statistical analysis.** The Student's t-test and one-way ANOVA were used to statistically analyze the data using SPSS 20.0 statistical software. The data from triplicate experiments are expressed as the mean  $\pm$  SD and a  $p < 0.05$  was considered to indicate a statistically significant result.

## Results

**In vitro anticancer effects of OCT.** SGC-7901 cells were incubated with increasing concentrations of OCT for 24 h and the viability was evaluated by MTT assay (Fig. 1). Four different concentrations of OCT (1, 10, 100 and 1,000 nmol/l) inhibited the growth of SGC-7901 cells to varying extents. Considering that the efficiency of inhibition for 10 and 100 nmol/l were comparable, 10 nmol/l of OCT was chosen as the effective concentration for the present study.

**OCT decreases p-Akt levels and increases ZAC expression.** SGC-7901 cells were treated with 10 nmol/l of OCT for 24 h. Compared to the control group, no significant change in the

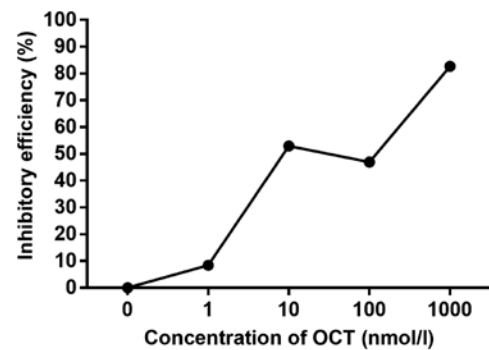


Figure 1. Inhibitory efficiency of SGC-7901 cells is determined at different concentrations of OCT using an MTT viability assay; 10 and 100 nmol/l OCT inhibited SGC-7901 cell growth to a similar extent, thus, 10 nmol/l was chosen as the representative concentration.

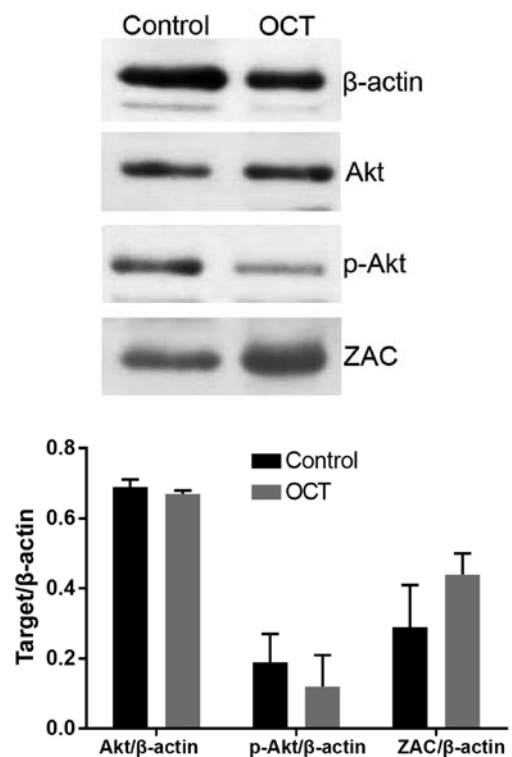


Figure 2. Effect of 10 nmol/l of OCT at 24 h on the expression levels of Akt, p-Akt and ZAC in SGC-7901 cells. There was no change in the expression of the Akt protein when compared to the control groups, although there was a decrease in the p-Akt level in the OCT-treated cells compared to the level in the control group. The expression of the ZAC protein in the OCT-treated cells was significantly increased when compared to the level in the control group ( $p < 0.05$ ).

expression of Akt was detected ( $p > 0.05$ ); however, the level of p-Akt was significantly decreased ( $p < 0.05$ ). In addition, the expression of ZAC was significantly increased in the OCT-treated cells when compared to that noted in the control group ( $p < 0.05$ ; Fig. 2). Overall, Akt levels were not affected by OCT, yet OCT decreased the amount of p-Akt and increased the levels of ZAC in the SGC-7901 cells.

**OCT increases P300-HAT activity in the gastric cancer cells.** The P300-HAT activity in the nucleoprotein of SGC-7901 cells was detected spectrophotometrically after incubation

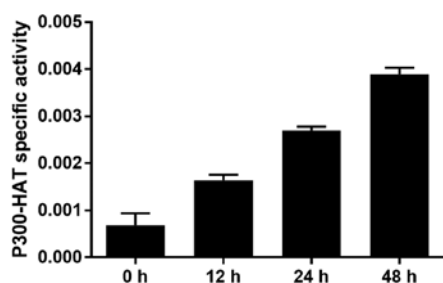


Figure 3. Spectrophotometric method was applied to detect the specific activity of P300-HAT in the SGC7901 cells treated with 10 nmol/l OCT. OCT increased P300-HAT activity in a time-dependent manner ( $p < 0.05$ ).

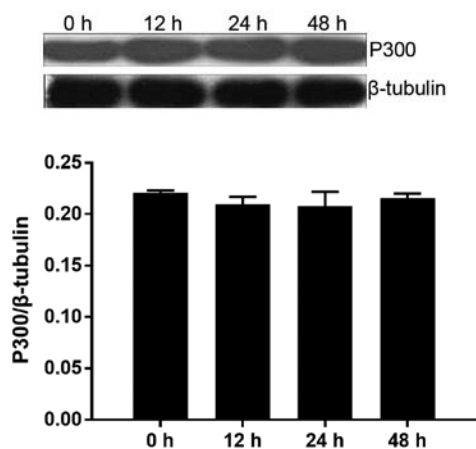


Figure 4. *P300* gene expression in 10 nmol/l OCT-treated cells remained constant at all time-points ( $p > 0.05$ ).

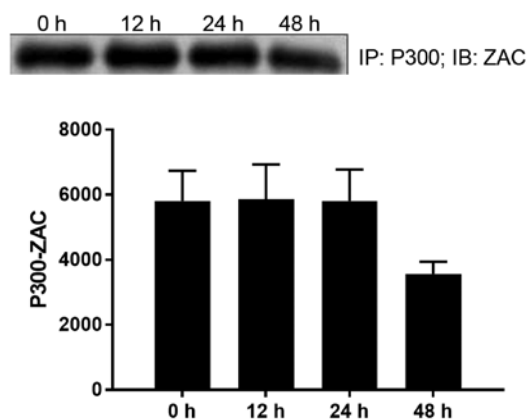


Figure 5. Interaction between ZAC and P300 in SGC-7901 cells. The amount of the ZAC-P300 complex did not change in the 10 nmol/l OCT-treated SGC-7901 cells at 0, 12 and 24 h ( $p > 0.05$ ); however, it decreased when treated with OCT for 48 h ( $p < 0.05$ ).

with OCT for 12, 24 and 48 h. OCT (10 nmol/l) significantly enhanced P300-HAT activity in the SGC-7901 cells in a time-dependent manner ( $p < 0.05$ ; Fig. 3). This outcome revealed that OCT increased the overall activity of P300-HAT in the SGC-7901 cells.

**OCT does not alter *P300* gene expression in the gastric cancer cells.** To observe the effects of OCT on *P300* gene expression, the amount of P300 in SGC-7901 cells was determined using

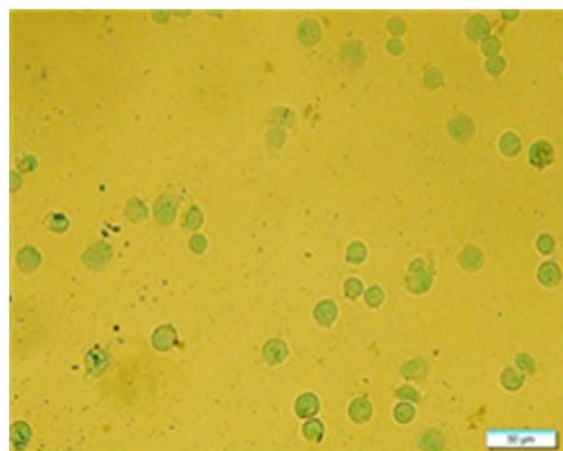


Figure 6. Representative micrograph of the appearance of pS10-H3 IR after 12 h of 10 nmol/l of OCT treatment. The pS10-H3 IR appeared as brown granules and was mainly distributed in the periphery of the nucleus.

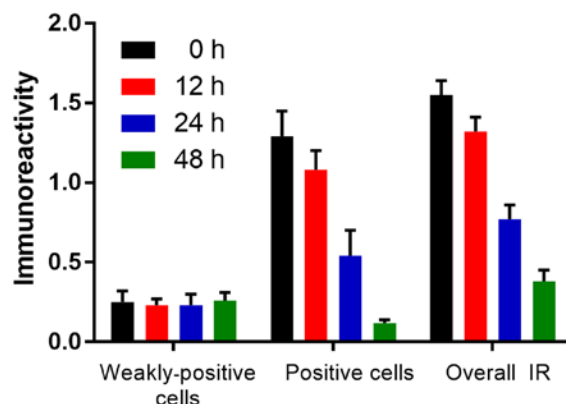


Figure 7. Effect of OCT on pS10-H3 IR in the SGC-7901 cells. There was no difference between the average pS10-H3 IR across all weakly positive cell conditions. The averages of pS10-H3 IR for positive cells were decreased in a time-dependent manner.

western blotting. There was no significant difference in *P300* gene expression between the control and the experimental groups when SGC-7901 cells were incubated with OCT for 12, 24 and 48 h ( $p > 0.05$ ; Fig. 4). This result demonstrated that the *P300* gene expression was not altered by OCT treatment in the SGC-7901 cells.

**Interaction between ZAC and P300 in the gastric cancer cells.** The co-immunoprecipitation assay showed that ZAC interacted with P300 in the SGC7901 cells. There was no significant difference in the amount of ZAC-P300 complex after 12 and 24 h of treatment with 10 nmol/l of OCT when compared to the control group ( $p > 0.05$ ); however, the amount of ZAC-P300 at 48 h was significantly decreased ( $p < 0.05$ ; Fig. 5). Overall, the interaction between ZAC and P300 remained constant in the SGC-7901 cells without OCT treatment and in those treated with 10 nmol/l of OCT for 12 and 24 h.

**OCT decreases pS10-H3 IR in positive gastric cancer cells.** The IR of pS10-H3 in SGC-7901 cells was determined using immunocytochemistry. pS10-H3 IR appeared as brown

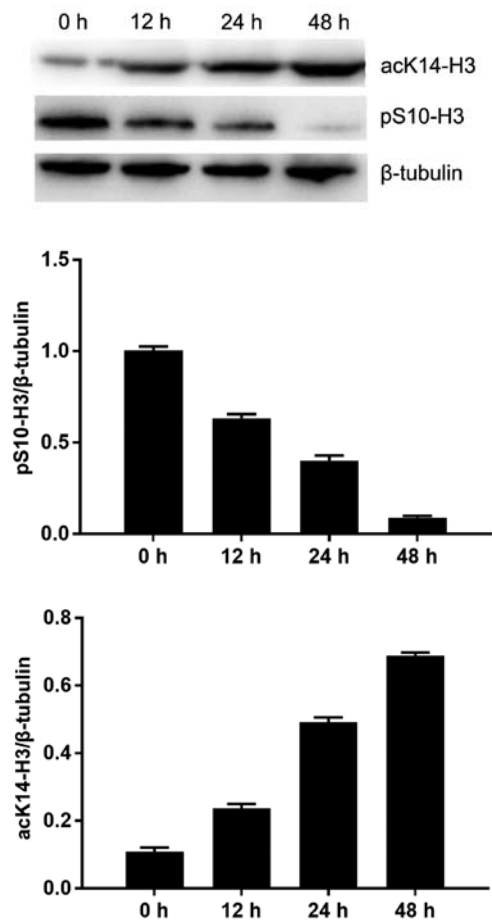


Figure 8. OCT (10 nmol/l) decreased the level of pS10-H3 ( $p<0.01$ ) and increased the level of acK14-H3 ( $p<0.01$ ) in the SGC-7901 cells in a time-dependent manner.

granules and was mainly distributed in the periphery of the nucleus (Fig. 6). The average percentage of weakly positive and positive cells in each group was calculated. The average IR of pS10-H3-positive cells decreased in a time-dependent manner. However, there was no significant difference ( $p>0.05$ ) in the average IR of weakly positive cells between the control and experimental groups (Fig. 7). Thus, OCT decreased pS10-H3 IR levels in the SGC-7901-positive cells.

**OCT decreases the level of pS10-H3 in the gastric cancer cells.** To determine the effect of OCT on pS10-H3, the amount of pS10-H3 in SGC-7901 cells was quantified using western blotting. The relative level of pS10-H3 was significantly decreased in a time-dependent manner in cells treated with 10 nmol/l of OCT ( $p<0.01$ ). Therefore, OCT significantly decreased the level of pS10-H3 in the SGC-7901 cells (Fig. 8).

**OCT increases acK14-H3 IR in gastric cancer cells.** Similar to the pS10-H3 IR, the acK14-H3 IR appeared as brown granules and was mainly distributed in the periphery of the nucleus (Fig. 9). The average level of acK14-H3 IR for weakly positive and positive cells in the control group was significantly lower than that in the experimental groups ( $p<0.01$ ; Fig. 10). OCT was therefore able to significantly increase acK14-H3 IR in the SGC-7901 cells.

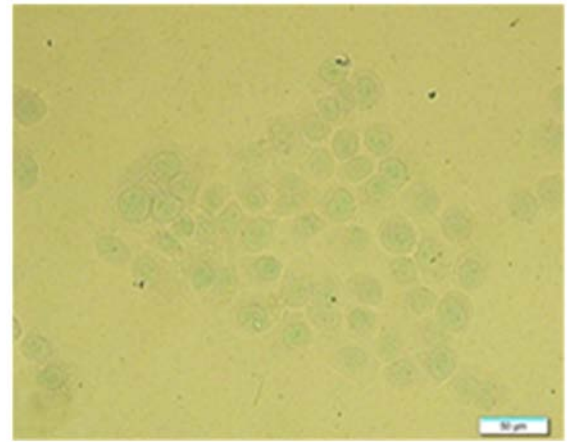


Figure 9. A representative micrograph of the appearance of acK14-H3 IR after 48 h of 10 nmol/l OCT treatment. acK14-H3 IR appeared as brown granules and was mainly distributed in the periphery of the nucleus.

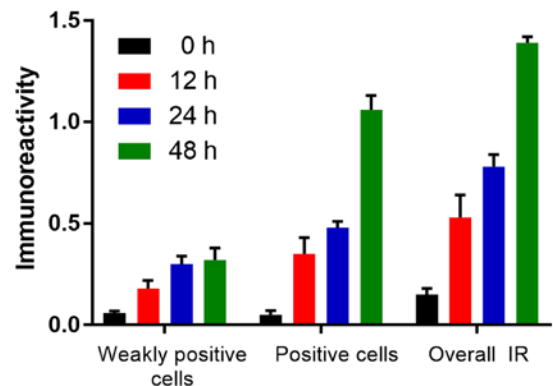


Figure 10. The average level of acK14-H3 IR in the weakly positive and positive cells in the control group was significantly lower than that in all OCT-treated groups ( $p<0.01$ ).

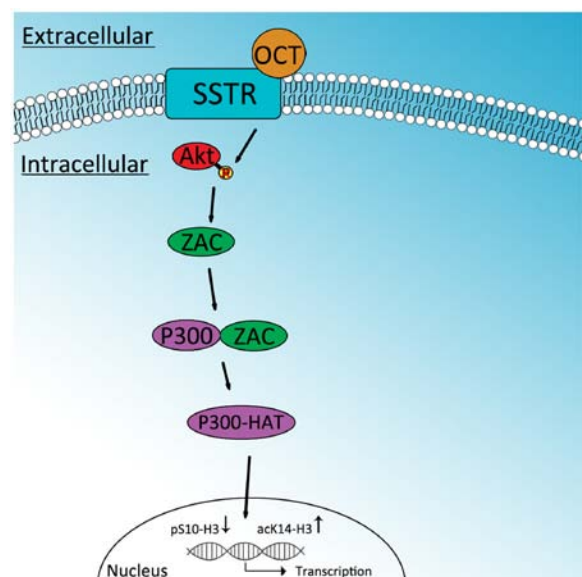


Figure 11. Proposed mechanism by which OCT acts to inhibit SGC-7901 cell proliferation. OCT binds to the SSTR to lower p-Akt and increase ZAC expression. ZAC interacts with P300 to increase P300-HAT activity which acts in the nucleus to decrease pS10-H3 (decreases the expression of proliferation-promoting genes) and increase acK14-H3 (increases the expression of proliferation-inhibiting genes).

*OCT increases the level of acK14-H3 in the gastric cancer cells.* To determine the effect of OCT on acK14-H3, the amount of acK14-H3 in the SGC-7901 cells was determined using western blotting. The level of acK14-H3 was significantly increased in a time-dependent manner in cells treated with 10 nmol/l of OCT ( $p < 0.01$ ). OCT therefore significantly increased the level of acK14-H3 in the SGC-7901 cells (Fig. 8).

## Discussion

The present study examined the mechanism of OCT in the inhibition of SGC-7901 cell proliferation. Since the inhibitory efficiency of 10 and 100 nmol/l of OCT were similar, 10 nmol/l of OCT was selected as the effective concentration (Fig. 1).

Gao *et al* suggested that Akt/PKB and telomerase activity in SGC-7901 cells was significantly inhibited when the cells were exposed to 1  $\mu$ g/ml of OCT for 12, 24 and 48 h compared to that of the control counterparts (23). The present study demonstrated that there was no difference in the level of Akt between the OCT-treated group and that of the control group in SGC-7901 cells. However, there was a decrease in the p-Akt level in the SGC-7901 cells treated with 10 nmol/l of OCT for 24 h compared to the control group (Fig. 2); this suggested that OCT inhibited Akt activity rather than Akt expression. This decrease in Akt activity may shift SSTR signaling from pro-survival to promoting the inhibition of SGC-7901 cell growth.

In pituitary tumor cells, OCT produces its antiproliferative action by acting on the PI3K/Akt signaling pathway and by increasing *ZAC1* gene expression (24). It has been previously shown that there is a positive correlation between the expression of ZAC and SST in gastric cancer tissue. Through the combination of SST and the SSTR3, ZAC expression was promoted and resulted in the inhibition of gastric tumor cell growth (15). Our present study revealed that OCT upregulated the expression of ZAC in SGC-7901 cells (Fig. 2) which supports the notion that OCT inhibits the proliferation of gastric cancer cells by reducing the level of Akt phosphorylation and upregulating ZAC expression.

Our research demonstrated that the P300-HAT activity in the SGC-7901 cells was increased in a time-dependent manner after being incubated with 10 nmol/l of OCT (Fig. 3); this indicated that OCT upregulated the activity of P300-HAT. The mechanism by which OCT upregulates P300-HAT activity in SGC-7901 cells is potentially multi-faceted. Firstly, OCT increases the activity of P300-HAT by upregulating the expression of P300 in SGC-7901 cells. Secondly, since OCT upregulates the expression of ZAC in SGC-7901 cells, ZAC and P300 proteins can interact to increase P300-HAT activity. Thirdly, increased P300 and ZAC have a synergistic action on the activity of P300-HAT. Our results revealed that there was no difference in the *P300* gene expression between the control and the experimental groups (Fig. 4), therefore, the first and the third hypotheses can be excluded. In light of this, we applied co-immunoprecipitation to observe the interaction of ZAC and P300 protein in SGC-7901 cells.

Coordinated binding of ZAC zinc fingers and C terminus to P300 regulates HAT function by increasing histone and acetyl coenzyme A affinities and catalytic activity. This concerted regulation of HAT function is mediated via the KIX and

CH3 domains of P300 in an interdependent manner. Notably, ZAC zinc fingers 6 and 7 simultaneously play key roles in DNA binding and P300 regulation (25). We suggested that P300-ZAC was present in the control and experimental groups by way of co-immunoprecipitation within SGC-7901 cells. Notably, there was no significant difference in the amount of P300-ZAC between the control and the 12 and 24-h groups. However, the amount of P300-ZAC in the 48-h group was significantly less than that in the other three groups (Fig. 5).

There are many potential reasons as to why the amount of P300-ZAC decreased at 48 h of OCT treatment. ZAC regulates the activities of the nuclear receptor, different members of the p53 family and all of the proteins that are crucial regulatory factors for cell growth, differentiation, equilibrium and development (26). The formation of a complex between P300 and p53 can, in turn, activate p53 (27,28). The activity of P300-HAT was found to be significantly increased when it interacted with special transcription factors (29). P300/CBP not only catalyzes the acetylation of all of the four core histones, but it also acetylates 70 other proteins and itself (10). Therefore, we speculated that OCT would upregulate the expression of the ZAC gene in SGC-7901 cells and that the interaction of ZAC and P300 not only would upregulate the activity of P300-HAT, but also influence the expression and activity of other transcription factors which can competitively inhibit the formation of ZAC-P300. This may thereby create a negative feedback loop, causing the amount of ZAC-P300 in SGC-7901 cells to decrease after OCT treatment for 48 h. However, the combination of these transcription factors and P300 may also increase the activity of P300-HAT, which can explain why ZAC plays a key role in inhibiting the pathway of gastric cancer cell proliferation. Our previous research demonstrated that 10 nmol/l of OCT, in the time period of 12–48 h, induced the expression of the ZAC gene in a time-dependent manner in gastric cancer cells (16). The result of the present study also showed that, in the time periods of 12–48 h, 10 nmol/l of OCT increased the activity of P300-HAT in the gastric cancer cells in a time-dependent manner (Fig. 3). Since OCT upregulates the activity of P300-HAT, it most likely affects the histone acetylation of ZAC and other tumor-suppressor gene promoters in SGC-7901 cells. ZAC may then affect histone acetylation of other tumor-suppressor gene promoters by the upregulation of P300-HAT activity, in addition to inducing apoptosis and cell cycle arrest. The increased expression of these genes may likely inhibit the proliferation of SGC-7901 cells.

Since tumor cells are heterogeneous, we observed the effect of OCT on pS10-H3 IR in SGC-7901 cells using an immunocytochemistry method. The levels of pS10-H3 IR in each cell were not uniform which indicates the functional status of heterogeneity in SGC-7901 cells. The results showed that pS10-H3 IR was mainly distributed in the periphery of the nucleus (Fig. 6), which may relate to the translocation of p-Akt from the cytoplasm to the nucleus. Our results revealed that the average IR of pS10-H3-positive cells was decreased compared to that of the control group, however, there was no significant difference between all groups for the average IR of weakly positive pS10-H3 cells (Fig. 7). This indicates that OCT mainly decreased the expression of highly active proliferation-promoting genes (positive cells). There was, however, little effect on proliferation-inhibiting genes that were being subtly



expressed (weakly positive cells) which further describes the heterogeneity of pS10-H3 expression in SGC-7901 cells.

In mammalian cells, pS10-H3 effects and causes stress of the stimulation of certain genes, such as *c-fos* and *c-jun*, via the mitogen stimulation signaling pathway (30,31). Our current results showed that OCT significantly decreased pS10-H3 (Fig. 8), which indicates that it may downregulate the mitogen stimulation signaling pathway. The expression of some proliferation-promoting genes may therefore be decreased by the observed decrease in pS10-H3.

Acetylated DNA is usually labeled as active chromatin. Lieberman-Aiden *et al* showed that the genome is divided into open (active) and closed (inert) states (32). The present study, revealed that acK14-H3 IR was mainly distributed in the periphery of the nucleus (Fig. 9) which seems contrary to the view of inert chromatin being distributed in the periphery of the nucleus (33). However, it has been shown that epigenetic modification can occur in chromatin located in the peripheral area of the nucleus in tumor cells to alter the three-dimensional structure of chromatin, rendering it active (34,35). The abrogation of the ability of the tumor cells to alter between open and closed states of chromatin may be related to the finding that acK14-H3 IR was mainly distributed in the periphery of the nucleus. The level of acK14-H3 IR between weakly positive and positive cells was not uniform which indicated that the state of chromatin in the SGC-7901 cells could be both open or closed. Both acK14-H3 IR (Fig. 10) and acK14-H3 (Fig. 8) increased in a time-dependent manner in the SGC-7901 cells treated with 10 nmol/l of OCT. Moreover, the IR results indicated that OCT may increase acK14-H3 in cells with a high expression of proliferation-inhibiting genes (positive cells), and have a smaller effect in cells with a low expression of proliferation-promoting genes (weakly positive cells), thereby inhibiting the growth of SGC-7901 cells.

All the results indicate that OCT may affect pS10-H3 and acK14-H3 in SGC-7901 cells to balance the expression of genes involved in proliferation and inhibition. OCT can significantly decrease pS10-H3 and significantly increase acK14-H3, which appears to be contrary to the view of mutual promotion between pS10-H3 and acK14-H3. This may be due to the fact that histone modifications have a high degree of background dependence. Histone PTMs may play a role in a gene-signal-specificity manner and do not have a universal code, instead, they are annotated against various regulatory signals within the genome (36). The biological outcomes of certain PTMs are usually dependent on the modification of chromatin and the cell background (37). Histone PTMs behave with a less strict 'code' and more with a complex 'language', which better illustrates that it is more reliant on the importance of the context, rather than convention. Only the 'language' deciphered in a certain context can produce a specific functional outcome (38).

There are, however, some limitations to the double modification of pS10-H3 and acK14-H3 as they may not be suitable for the expression of some genes. P300 is a cofactor of oncoproteins (such as *fos*, *jun* and *myb*) and transforming virus proteins (such as E1A), in addition to being a cofactor for tumor-suppressor proteins (such as p53, E2F, Rb or BRCA1 (39). By upregulating P300-HAT activity, OCT may also affect the expression of oncogenes in SGC-7901 cells. In

addition, cancer cells express multiple SSTTR subtypes which indicate that these receptors are coupled with other intracellular receptor systems and extracellular signal cascades (40). There is still a great deal of information on SSTTR subtypes pertaining to their involvement in physiological functions and different kinase and phosphatase activities that are still not fully understood.

In conclusion, the present study demonstrated that OCT may inhibit the proliferation of SGC-7901 cells by decreasing p-Akt, which in turn, increases ZAC. ZAC then interacts with P300 to increase P300-HAT activity. This ultimately decreases pS10-H3 and increases acK14-H3. Therefore, a decrease in pS10-H3 and an increase in acK14-H3 may be involved in the antiproliferative effects of OCT in SGC-7901 cells (Fig. 11).

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