An inhibitor of the acetyltransferases CBP/p300 exerts antineoplastic effects on gastrointestinal stromal tumor cells

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Abstract. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasm featured by activated mutations of KIT and PDGFRA. Although overall survival rates have greatly improved by the development of receptor tyrosine kinase inhibitors, most patients ultimately acquire resistance due to secondary mutations of KIT or PDGFRA. Inhibition of the histone acetyltransferases (HATs) CREB-binding protein (CBP) and p300 results in antineoplastic effects in various cancers. To determine whether CBP/p300 can serve as an antineoplastic target for GISTs, specific short interfering RNA sequences and the selective HAT inhibitor C646 were administered to GIST882 cells. Cell viability, apoptosis and the cell cycle were analysed using the Cell Counting Kit-8, a caspase-3/7 activity assay or Annexin V-fluorescein isothiocyanate/propidium iodide (PI) staining and PI staining. Gene and protein expression levels were measured by quantitative real-time polymerase chain reaction and western blotting, respectively. Transcriptional blockage of CBP rather than p300 resulted in suppression of cell proliferation. Interestingly, both CBP and p300 depletion enhanced caspase-3/7 activity. A lack of CBP and p300 caused ETS translocation variant 1 (ETV1) downregulation and KIT inhibition in GIST cells. Nevertheless, the absence of CBP, not p300, leads to extracellular signal-regulated kinase 1/2 inactivation and c-Jun NH₂-terminal kinase activation, suggesting a more crucial role for CBP than p300 in cell proliferation and survival. Furthermore, proliferation of GIST cells was reduced by administration of C646, a selective HAT inhibitor for CBP/p300. Apoptosis induction and cell cycle arrest were detected after exposure to C646, indicating that its antitumor activities were supported by its antiproliferative and proapoptotic effects. Additionally, C646 treatment attenuated ETV1 protein expression and inactivated KIT-dependent pathways. Taken together, the present study suggests that CBP/p300 may serve as novel antineoplastic targets and that use of the selective HAT inhibitor C646 is a promising antitumor strategy for GISTs.

Introduction

Gastrointestinal stromal tumors (GISTs), the most common mesenchymal neoplasms of the gastrointestinal tract (1), are characterized by gain-of-function mutations of the KIT or PDGFRA genes (2,3). They most likely originate from the interstitial cells of Cajal (ICC) or Cajal-like precursor cells whose immunohistochemical marker is CD117 (4,5). In recent years, the receptor tyrosine kinase (RTK) inhibitor imatinib mesylate (Gleevec; Novartis Pharmaceuticals Corp., East Hanover, NJ, USA), which targets the KIT and PDGFRA oncoproteins, has frequently been used to treat patients with advanced GISTs or has been used as an adjuvant agent (6). Although up to 70% of GIST patients respond to imatinib well at first, ~15% of patients may develop primary resistance, and most responding patients will eventually develop secondary resistance and have disease progression (7). Thus, novel therapeutic targets and effective antineoplastic strategies are still needed.

Primary resistance is mainly caused by KIT exon 9 mutations and the lack of activating KIT mutations, resulting in resistance to imatinib. The PDGFRA mutation D842V on exon 18 also contributes to primary resistance in GISTs (8). Acquired secondary mutations in the kinase domains of KIT or PDGFRA will lead to the development of imatinib resistance in most patients (9). Moreover, it has been reported that stem cell factor (SCF), a KIT ligand, may contribute to the acquired imatinib resistance (10). Although an increasing number of new-generation RTK inhibitors, such as sunitinib, nilotinib, vatalanib and masatinib, have demonstrated their antineoplastic activities in GISTs (11), the therapeutic effect varies depending on the pattern of genetic alterations.
Therefore, it is imperative that novel molecular targets and selective agents be developed and investigated to revolutionize antitumor strategies.

CREB-binding protein (CBP) and p300 (collectively referred to as CBP/p300) are transcriptional co-activators that integrate and maintain various gene regulator pathways and protein acetylation events with intrinsic histone acetyltransferase (HAT) activity (12). Over the last few years, their biological and pathological functions have been under extensive investigation. CBP/p300 play pivotal roles in differentiation, apoptosis and the cell cycle (13,14). However, there is a distinct relationship between CBP/p300 activity and tumorigenesis and malignancy. CBP/p300 inactivation will not only inhibit the growth of prostate cancer cells and melanoma cells (15,16) but will also induce apoptosis and cell cycle arrest in leukaemia cells (17). Interestingly, it has been illustrated that the co-activators CBP and p300 interact with an oncoprotein ETS translocation variant 1 (ETV1) (18), a distinctive transcription factor that is overexpressed in most prostate cancers (19). In addition, p300 directly acetylates ETV1 and thereby enhances its stability, DNA-binding capacity, and transcriptional activity in vitro (20). Recent studies have demonstrated that ETV1 is a specific survival factor that cooperates with KIT in GISTs, and our previous findings showed that ETV1 was highly upregulated within tumor tissues in conjunction with KIT expression (21,22). Therefore, CBP/p300 may play a vital role in tumorigenesis and progression of GISTs by regulating the functions of ETV1 and KIT-dependent pathways, serving as promising targets for antineoplastic therapy.

Recently, a small molecule inhibitor targeting CBP/p300, C646, has gained much attention for its anticancer potential. C646 administration inhibits histone acetylation and cell growth with relatively high selectivity and potency, suggesting its application as a prospective anticancer agent (23). Although the antitumor activity of C646 has been investigated in other tumors (15,17), its effects on GISTs have not yet been assessed. Here, we explored the therapeutic relevance of C646 administration inhibiting histone acetylation and cell growth in the homozygous KIT exon 13 K642E (KIT-exon13) mutation was a generous gift from Dr Jonathan Fletcher (Dana-Farber Cancer Institute, Boston, MA, USA). GIST882 cells were grown in RPMI-1640 medium (Corning Inc., Corning, NY, USA) with 15% fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin. The GIST cells were maintained in a humidified incubator at 37˚C in a 5% CO2 atmosphere. The CBP/p300 inhibitor, C646, was purchased from Selleck Chemicals LLC (Shanghai, China).

Materials and methods

Cell culture and reagents. The GIST882 cell line carrying the homozygous KIT exon 13 K642E (KIT-exon13) mutation was a generous gift from Dr Jonathan Fletcher (Dana-Farber Cancer Institute, Boston, MA, USA). GIST882 cells were grown in RPMI-1640 medium (Corning Inc., Corning, NY, USA) with 15% fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin. The GIST cells were maintained in a humidified incubator at 37˚C in a 5% CO2 atmosphere. The CBP/p300 inhibitor, C646, was purchased from Selleck Chemicals LLC (Shanghai, China).

Transfection of short interfering RNA (siRNA). To determine the role of CBP/p300 in GIST proliferation and apoptosis, GIST882 cells were cultured in 6- and 96-well plates until 70-80% confluence, respectively. siRNA transfections were performed with Lipofectamine RNAiMAX reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) in antibiotics-free medium according to the manufacturer’s instructions. GIST882 cells were transfected with 10 nmol/l siRNA. The control siRNA and siRNA against CBP and p300 were purchased from Santa Cruz Biotechnology, Inc. (Dallas, Texas, USA).

Cell proliferation assay. GIST882 cells (1.5x10^5/well) were seeded in 96-well plates and further cultured for 48 h before C646 and siRNA were added. After administration of C646 (for 24 and 48 h) or transfection with siRNA (for 24, 48 and 72 h), 10 µl of Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan) solution was added to each well, followed by incubation for 2 h at 37˚C. The optical density (OD) was measured at 450 nm with a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The mean OD values from 4-wells for each treatment were used as the index of cell viability. All experimental points were replicated in three plates. Cell viability was calculated using the following formula: OD value of each set/OD value of control group (DMSO control and blank control, respectively) x 100.

Caspase activity assay. GIST882 cells (1.5x10^5/well) were cultured in a 96-well plate for 48 h before being transfected with siRNA or treated with C646. After transfection with siRNA (for 24, 48 and 72 h) or administration of C646 (for 24 and 48 h), caspase-Glo 3/7 reagent (Promega, Madison, WI, USA) was added to each well at a ratio of 1:1 following the manufacturer's instructions. The luminescence intensity of each sample was measured in a plate-reading lumimeter (Promega). Caspase activity was expressed as a percentage of the control level [blank and dimethyl sulfoxide (DMSO) controls, respectively]. Luminescence intensity was measured in quadruplicate, and all experimental points were replicated in three plates.

Cell apoptosis assay. Apoptosis induction was detected using an Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis assay kit (Invitrogen Life Technologies). GIST882 cells (7x10^4/well) were plated on 6-well plates and further cultured for 48 h before being treated with C646. After incubation for 24, 48 or 72 h, the cells were harvested by trypsin without EDTA and centrifuged at 200 x g for 5 min. The cell pellets were resuspended in 100 µl of 1X binding buffer. Next, 5 µl of Annexin V-FITC and 1 µl of PI were added and mixed well. After incubation for 15 min in the dark, 400 µl of 1X binding buffer was added to each sample. The stained cells were evaluated by flow cytometry (NovoCyte; ACEA Biosciences, Inc., San Diego, CA, USA), and the data were analysed using FlowJo 7.6.2 software (Tree Star, Inc., Ashland, OR, USA). All experimental sets were conducted in triplicate.

Cell cycle assay. Cell cycle analysis was performed using a Cell Cycle kit purchased from Multi Sciences (Lianke) Biotech Co., Ltd. (Hangzhou, China). GIST882 cells (7x10^5/well) were seeded in 6-well plates and further cultured for 48 h before being treated with C646. After administration of C646 for 24, 48 or 72 h, the cells were trypsinized and collected by centrifugation at 200 x g for 5 min. The cells were then fixed with 75% ethanol at -20˚C overnight and rehydrated with
ice-cold PBS for 15 min before being stained with 1 ml of DNA staining solution. The stained cells were then evaluated by flow cytometry as described above, and the data were analysed using ModFit LT V3.3.11 software (Verity Software House, Inc., Topsham, ME, USA). All experimental sets were conducted in triplicate.

**Quantitative real-time polymerase chain reaction (PCR).** According to the transfection protocol, GIST882 cells were divided into four groups: blank, negative control (NC), sip300 and siCBP. The total RNA of each sample was extracted using TRIzol reagent (Invitrogen Life Technologies) following the manufacturer's instructions. Total RNA (2 μg) was reverse-transcribed using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Biotechnology, Co., Ltd., Dalian, China). DNA products were amplified with SYBR® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Biotechnology, Co., Ltd.). The primer sequences were as follows: forward, 5'-CCTGAG TAGGGGCAACAAGA-3' and reverse, 5'-GTGTCTCCACA TGGTGCTTGT-3' for human p300; forward, 5'-CTGCAAC GACATGACT-3' and reverse, 5'-GAAAGTGACCATTCTTG TTG-3' for human CBP; and forward, 5'-AGAAGGGCTGGG CTGCTATATGTGGA-3' and reverse, 5'-AGGGGCGATCCACA GCTTCT-3' for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Real-time PCR was performed using a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The thermocycling conditions were 10 min at 95°C as the initial denaturation step, followed by 40 cycles at 95°C for 30 sec and 60°C for 34 sec. Gene expression levels were measured using the threshold cycle (Ct) value, and relative fold-expression changes were normalized to GAPDH amplification. The 2^(-ΔΔCt) method was used to measure the relative changes in gene expression.

**Western blotting.** Total proteins were harvested from GIST882 cells using cell lysis buffer (Cell Signaling Technology, Inc., Beverly, MA, USA) according to the manufacturer's instructions. Equal amounts of protein were applied to 8-12% gels and were subjected to SDS-PAGE. The samples were divided into four groups: blank, negative control (NC), sip300 and p300. The total RNA of each sample was extracted using TRIzol reagent (Invitrogen Life Technologies) following the manufacturer's instructions. Total RNA (2 μg) was reverse-transcribed using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara Biotechnology, Co., Ltd., Dalian, China). DNA products were amplified with SYBR® Premix Ex Taq™ (Tli RNaseH Plus) (Takara Biotechnology, Co., Ltd.). The primer sequences were as follows: forward, 5'-CCTGAG TAGGGGCAACAAGA-3' and reverse, 5'-GTGTCTCCACA TGGTGCTTGT-3' for human p300; forward, 5'-CTGCAAC GACATGACT-3' and reverse, 5'-GAAAGTGACCATTCTTG TTG-3' for human CBP; and forward, 5'-AGAAGGGCTGGG CTGCTATATGTGGA-3' and reverse, 5'-AGGGGCGATCCACA GCTTCT-3' for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Real-time PCR was performed using a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The thermocycling conditions were 10 min at 95°C as the initial denaturation step, followed by 40 cycles at 95°C for 30 sec and 60°C for 34 sec. Gene expression levels were measured using the threshold cycle (Ct) value, and relative fold-expression changes were normalized to GAPDH amplification. The 2^(-ΔΔCt) method was used to measure the relative changes in gene expression.

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**CBP/p300 downregulation enhances caspase-3/7 activity in GIST882 cells.** In the present study, caspase-3/7 activity was measured to evaluate apoptosis induction following CBP/p300 downregulation. As shown in Fig. 1F, consistent with the antiproliferative effects induced by CBP silencing, caspase-3/7 activity in GIST882 cells was markedly enhanced 3.5-fold compared with the negative control after transfection for 72 h (P<0.01). Notably, inhibition of p300 by siRNA also increased caspase-3/7 activity 2.2-fold, which was much higher than that in the negative control group after transfection for 72 h (P<0.01).

**CBP/p300 silencing results in inhibition of ETV1 and KIT-dependent pathways and activation of apoptotic pathways.** To elucidate the molecular mechanisms involved in the antineoplastic effects on GIST882 cells, we detected ETV1 protein levels and KIT-dependent pathway regulation following siRNA transfection for 72 h. As shown in Fig. 2, both CBP and p300 inhibition caused considerable ETV1 degradation compared with the negative control. Phosphorylation of KIT decreased considerably as CBP and p300 were downregulated, in spite of their high homology. CBP/p300 downregulation enhances caspase-3/7 activity in GIST882 cells. In the present study, caspase-3/7 activity was measured to evaluate apoptosis induction following CBP/p300 downregulation. As shown in Fig. 1F, consistent with the antiproliferative effects induced by CBP silencing, caspase-3/7 activity in GIST882 cells was markedly enhanced 3.5-fold compared with the negative control after transfection for 72 h (P<0.01). Notably, inhibition of p300 by siRNA also increased caspase-3/7 activity 2.2-fold, which was much higher than that in the negative control group after transfection for 72 h (P<0.01).
of cell proliferation and caspase-3/7 activation. Therefore, we next investigated whether C646, a selective CBP/p300 inhibitor, was able to exert similar effects. Its potential antineoplastic activity was validated by treating GIST882 cells with an increasing concentration of C646 (5-15 µmol/l) for 24 and 48 h, respectively. Moreover, imatinib (500 nmol/l) alone or in combination with C646 at the same doses (5-15 µmol/l) were used to assess the possible additive effect. As shown in Fig. 3A, C646 administration had strong inhibitory effects on cell proliferation in the GIST822 cells. The cell viability of GIST882 cells significantly decreased after treatment with imatinib combined with C646 (15 µmol/l) for 48 h compared with the group treated with 15 µmol/l C646 alone (P<0.01). To detect caspase-3/7 activity, C646 (1-15 µmol/l) alone or in combination with imatinib (500 nmol/l) was administered to GIST cells for 24 and 48 h, respectively. As shown in Fig. 3B, caspase-3/7 activity significantly increased after treatment with C646 (15 µmol/l) alone or in combination with imatinib (500 nmol/l) compared with DMSO control group (P<0.01). In the combination studies, caspase-3/7 activity significantly increased after treatment with imatinib combined with C646 (15 µmol/l) for 48 h compared with the group treated with 15 µmol/l C646 alone (P<0.01).

C646 exerts its antineoplastic effect via apoptosis induction and cell cycle arrest in GIST882 cells. To further assess the apoptosis induced by C646, Annexin V-FITC/PI staining and flow cytometry were performed. C646 administration induced significant apoptosis in GIST cells compared with DMSO control group (Fig. 4A). As shown in Fig. 4B, apoptotic cell death was induced in a dose-dependent manner, and 15 µmol/l C646 caused significant apoptosis in GIST cells compared with DMSO group (P<0.01). In addition, the apoptotic rate increased accordingly over time in GIST882 cells exposed to 15 µmol/l C646 (Fig. 4C). The effect of C646 on the cell cycle was examined via PI staining and flow cytometry. Our results indicated that C646 treatment led to an increase in GIST cells in the G1-phase, whereas the S-phase population declined considerably (Fig. 4D). Notably, 15 µmol/l C646 immediately induced cell cycle arrest at the G1-phase in GIST882 cells within 24 h; this inhibitory effect did not follow a dose- or time-dependent pattern (Fig. 4E and F).

C646 attenuates ETV1 expression and inactivates KIT-dependent pathways. To address the molecular mechanisms underlying the antineoplastic effects induced by C646 in GIST882 cells, the expression levels of ETV1 and functional changes in the KIT pathway were explored. The GIST cells
were treated with C646 (5-20 µmol/l) and imatinib (500 nmol/l) alone and in combination with C646 (15 µmol/l) and were further incubated for 24 h. As shown in Fig. 5, C646 administration strongly decreased ETV1 expression in a dose-dependent manner. Considerable inhibition of KIT phosphorylation was observed following C646 treatment. Moreover, the phosphorylation levels of Akt and ERK1/2 were attenuated accordingly after exposure of the cells to C646 for 24 h. In contrast, C646 had little effect on the expression levels of total Akt and ERK1/2. In the combination study, synergistic inhibitory effects on KIT and ERK1/2 activation were observed in GIST882 cells after treatment with comparatively low concentrations of imatinib (500 nmol/l) and C646 (15 µmol/l). Taken together, C646 may exert its antineoplastic effects through inhibition of ETV1 expression and inactivation of the KIT-dependent pathway, leading to suppressed phosphorylation of Akt and ERK1/2.

**Discussion**

Recent advances in the knowledge of GIST pathogenesis have contributed to the rapid developments of therapeutic agents targeting abnormalities in KIT and PDGFRA. Although the advent of potent RTK inhibitors, such as imatinib and sunitinib, has greatly improved median overall survival in inoperable and metastatic cases (24-26), most patients eventually develop resistance due to various resistance mechanisms (27,28), representing a clinical challenge to antitumor therapy. As secondary mutations have appeared in the majority of the patients who develop resistance (9), present antineoplastic strategies are aimed at improving the efficiency and selectivity of the anticancer therapy in spite of these mutations. Based on this goal, posttranslational modifications involving oncology and therapy have been intensively studied in GISTs over the past few decades. It has been suggested that microRNA-221/222 may serve as a new therapeutic strategy for treating TKI-resistant GIST (29). Here, we explore the functions of the HAT proteins CBP and p300, which are involved in GIST tumorigenesis. Being a part of the vital molecules in posttranslational modifications, the acetyltransferases CBP/p300 play crucial roles in oncogenesis and progression. CBP/p300 are known to have tumor-promoting effects as they have been shown to accelerate the progression of colon and prostate cancers (30,31). However, mutations of the CBP and p300 genes or dysfunction of the proteins will promote cell proliferation and consequently increase the incidence of haematological malignancies,

![Figure 2. CBP/p300 inhibition causes ETV1 inhibition, KIT-dependent pathway inactivation and apoptotic pathway activation. GIST882 cells were transfected with siRNAs targeting CBP and p300 for 72 h. Western blotting was performed to detect the expression levels of ETV1, KIT-dependent signalling pathway-related proteins and apoptotic pathway-related proteins. GAPDH was used as a reference. CBP, CREB-binding protein; ETV1, ETS translocation variant 1; GIST, gastrointestinal stromal tumor; siRNA, short interfering RNA; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.](image)

![Figure 3. The CBP/p300 inhibitor C646 induces suppression of cell proliferation and apoptosis. GIST882 cells were treated with C646 at doses ranging from 5 to 15 µmol/l for 24 or 48 h. Imatinib (500 nmol/l) alone or in combination with C646 at the same doses (5-15 µmol/l) were used to evaluate the possible synergistic effect. DMSO was used as the vehicle control. (A) Cell proliferation was measured using a CCK-8 assay (**P<0.01 vs. DMSO group treated for 48 h, ##P<0.01 vs. the group treated with 15 µmol/l C646 for 48 h). (B) Cellular apoptosis was assessed based on caspase-3/7 activity (**P<0.01 vs. DMSO group treated for 48 h, ##P<0.01 vs. the group treated with 15 µmol/l C646 for 48 h). CBP, CREB-binding protein; GIST, gastrointestinal stromal tumor; DMSO, dimethyl sulfoxide; CCK-8, Cell Counting Kit-8.](image)
suggesting a tumor-suppressor role of CBP/p300 in carcinogenesis (32,33). Thus, the function of CBP/p300 varies accordingly under different circumstances. The present study demonstrated that blockage of CBP and p300 by specific siRNAs induced cell growth suppression in GITS882 cells. Interestingly, whereas transcriptional inhibition of CBP exhibited a strong antiproliferative activity, p300 inhibition did not exert a similar inhibitory effect on cell proliferation, implying that CBP rather than p300 played a dominant role in the regulation of cell proliferation. These results support the idea that despite their high degree of homology, CBP and p300 may have non-overlapping functions and accordingly may play different roles (34). Nonetheless, silencing of CBP/p300 significantly enhanced caspase-3/7 activity, indicating that apoptosis induction did not completely parallel growth inhibition. Our results revealed that depletion of CBP/p300 resulted in decreased ETV1 expression levels along with KIT inactivation in GIST882 cells. Previous studies have reported that CBP/p300 directly interacted with ETV1 and enhanced its DNA-binding and transactivation through acetylation (20). Additionally, upregulated ETV1 expression was detected in ICC, which are precursors of GISTs (35). Therefore, CBP/p300 may represent a specific target for GISTs due to its role in regulating ETV1, the key transcriptional factor that is highly expressed in both GISTs and its precursor cells. These findings prompt us to hypothesize that CBP/p300 may stabilize ETV1 via direct acetylation and activate KIT-dependent pathways, contributing to cell proliferation and survival in GISTs.

Figure 4. C646 induces apoptosis and cell cycle arrest in GIST cells. GIST882 cells were exposed to C646 (5-25 µmol/l) for 24 to 72 h, respectively. DMSO was used as the vehicle control. (A-C) Apoptosis induced by C646 was assessed by Annexin V-FITC/PI staining and was further analysed by flow cytometry (*P<0.01 vs. DMSO group or the group treated with 15 µmol/l C646 at 0 h). (D-F) Cell cycle analysis was performed by flow cytometry to detect cell cycle arrest induced by C646 following PI staining (**P<0.01 vs. DMSO group or the group treated with 15 µmol/l C646 at 0 h). GIST, gastrointestinal stromal tumor; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; PI, propidium iodide.
The data reported herein indicate that CBP plays a key role in the regulation of ERK1/2 and JNK activities, whereas p300 does not act in a similar way. It is widely acknowledged that ERK1/2, a subtype of mitogen-activated protein kinases (MAPKs), regulates cell proliferation, differentiation and migration through KIT-dependent pathways (36). JNK, another subtype of MAPKs that is expressed but not phosphorylated in GISTS in vitro (37), participates in the death receptor signaling pathway required for apoptosis induction in various cancers (38-40). Therefore, CBP is required for tumor growth through ERK1/2 phosphorylation and apoptosis regulation via inactivation of JNK in GIST882 cells, whereas p300 does not appear to contribute to these activities. However, both CBP and p300 silencing led to the upregulation of proapoptotic Bax and the downregulation of antiapoptotic Bcl-2 and Bcl-xl, members of the Bcl-2 family that are involved in the mitochondrial cell death pathway (41), suggesting that CBP/p300 affect apoptosis through the regulation of Bcl-2 family proteins. Thus, CBP may regulate apoptosis via both the death receptor signaling pathway and the mitochondrial cell death pathway in GIST cells. In contrast, p300 affects apoptotic cell death through the mitochondrial cell death pathway and has little effect on the death receptor signaling pathway.

To date, a variety of histone deacetylase (HDAC) inhibitors have been shown to induce apoptosis in tumor cells and have been introduced into clinical trials (42). Although pharmacological inhibition of deacetylation by HDAC inhibitors can exert antiproliferative and proapoptotic effects on several GIST cell lines (43), not much is known regarding acetyltransferase inhibitors. To this end, the present study is the first to show strong antiproliferative and proapoptotic effects in imatinib-sensitive GIST cells by C646, a competitive inhibitor of HATs. The GIST882 cells appear to be highly sensitive to the HAT inhibitor based on the observation that GIST cell proliferation is reduced substantially following exposure to C646. Treatment with C646 resulted in marked apoptotic cell death, demonstrating potent antineoplastic activity. In addition to proapoptotic activity, C646 treatment also induced considerable cell cycle arrest at an early time-point (24 h), showing the high efficacy of its antigrowth effect. Moreover, C646 administration inhibited KIT phosphorylation and deactivated ERK1/2, disrupting the downstream cascade for tumor proliferation and survival. Overall, C646-induced antineoplastic effects follow a similar pattern as that of CBP downregulation. Therefore, it is conceivable that C646 is able to exert these antitumor effects mainly through inhibition of CBP activity. Our data further showed that imatinib and C646 combination therapy would exert synergistic inhibitory effects on GIST cells, suggesting a potential usage of C646 in GIST therapy especially for imatinib-resistant cases. This effect may be partly attributed to the inactivation of KIT and ERK1/2. Additionally, C646 exhibits a strong inhibitory effect on the phosphorylation of Akt, another downstream component of KIT and a critical molecule in GIST tumorigenesis (44). Taken together, our study indicates that C646 may be a promising antineoplastic reagent for GIST therapy.

However, this study has some limitations. Further investigations of the expression and function of CBP/p300 in clinical GIST samples or animal models are required to clarify its role in tumorigenesis and progression in vivo. Moreover, although an immediate synergism with imatinib was acquired following C646 administration in GIST882 cells, these effects require further examination in other GIST cell lines that are sensitive and resistant to imatinib. The efficacy and safety of C646 as an antineoplastic agent for GISTS have not yet been fully characterized and remain to be validated both in vitro and in vivo.

In conclusion, our results demonstrate that CBP silencing will inhibit cell proliferation and promote apoptosis induction, whereas p300 inhibition has little effect on cell growth, posing some advantages of CBP as a more effective target for GIST therapy. The molecular mechanisms underlying this antineoplastic effect may be attributed to ETV1 downregulation and inactivation of KIT-dependent pathways. CBP regulates apoptosis through both the death receptor pathway and the mitochondrial cell death pathway in GIST882 cells, whereas p300 has little effect on the death receptor pathway. Furthermore, the HAT inhibitor C646 attenuates ETV1 expression and inactivates KIT-dependent pathways, triggering antiproliferative effects in GIST cells and inducing apoptosis or cell cycle arrest. Consequently, the present study provides mechanistic insight and supports the notion that CBP/p300 inactivation by HAT inhibitors may serve as a prospective approach for antitumor therapeutics against GISTS.

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Figure 5. C646 attenuates ETV1 expression and inactivates KIT-dependent pathways. GIST882 cells were treated with C646 (5-20 µmol/l) for 24 h. Imatinib (500 nmol/l) alone or in combination with C646 (15 µmol/l) was used for the combination study. DMSO was used as the vehicle control. The expression levels of ETV1 and KIT-dependent pathway-related proteins were analysed by western blotting. GAPDH was used as a reference. ETV1, ETS translocation variant 1; GIST, gastrointestinal stromal tumor; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.