Abstract

8-Chloro-cyclic AMP (8-Cl-cAMP), which is known to induce growth inhibition, apoptosis, and differentiation in various cancer cell lines, has been studied as a putative anticancer drug. However, the mechanism of anticancer activities of 8-Cl-cAMP has not been fully understood. Previously, we reported that the 8-Cl-cAMP-induced growth inhibition is mediated by protein kinase C (PKC) activation. In this study, we found that p38 mitogen-activated protein kinase (MAPK) also plays important roles during the 8-Cl-cAMP-induced growth inhibition and apoptosis. SB203580 (a p38-specific inhibitor) recovered the 8-Cl-cAMP-induced growth inhibition and apoptosis, whereas other MAPK inhibitors, such as PD98059 (an extracellular signal-regulated kinase–specific inhibitor) and SP600125 (a c-Jun NH₂-terminal kinase–specific inhibitor), had no effect. The phosphorylation (activation) of p38 MAPK was increased in a time-dependent manner after 8-Cl-cAMP treatment. Furthermore, SB203580 was able to block PKC activation induced by 8-Cl-cAMP. However, PKC inhibitor (GF109203x) could not attenuate p38 activation, indicating that p38 MAPK activation is upstream of PKC activation during the 8-Cl-cAMP-induced growth inhibition. 8-Chloro-adenosine, a metabolite of 8-Cl-cAMP, also activated p38 MAPK and this activation was blocked by adenosine kinase inhibitor. These results suggest that 8-Cl-cAMP exerts its anticancer activity through p38 MAPK activation and the metabolite(s) of 8-Cl-cAMP mediates this process. (Cancer Res 2005; 65(11): 4896-901)

Introduction

Cyclic AMP (cAMP) is a well-known secondary messenger that triggers various cellular physiologic responses, such as cell growth and proliferation (1), regulation of metabolism (2), gene induction (3, 4), and ion channel regulation (5). These biological actions of cAMP seem to be cell type specific. For example, cAMP can stimulate cell growth in many cell types, such as PC12 cells, preadipocytes, and Sertoli cells (6–8), whereas it inhibits cell growth in other cells, such as NIH3T3 and adipocytes (9, 10). Classically, cAMP has been known to provoke its cellular responses through protein kinase A (PKA) activation. After binding of cAMP to the regulatory (R) subunits of PKA, R subunits are dissociated from the holoenzyme and catalytic (C) subunits are activated. Although this cAMP-PKA signaling is a well-established pathway, another cAMP signal transducer has been recently identified, the exchange protein directly activated by cAMP-Rap1 pathway (11, 12). This exchange protein directly activated by cAMP-Rap1 signal can regulate cellular growth by modulating the activities of mitogen-activated protein kinases (MAPK; ref. 13).

8-Chloro-cAMP (8-Cl-cAMP), one of the cAMP analogues, induces inhibition of cellular proliferation and apoptosis when treated to various cancer cell lines (14). Due to its antitransforming activities, 8-Cl-cAMP has been tested as a cancer therapeutic drug (15, 16). However, the signaling mechanisms that govern the 8-Cl-cAMP-induced growth inhibition are still uncertain. Cho-Chung et al. suggested that differential regulation of PKA isozymes, PKA type I and II, is the major cause of the 8-Cl-cAMP-induced anticancer activity (17). When treated to cancer cells, 8-Cl-cAMP selectively down-regulates type I regulatory (R) subunit; then, the decreased ratio of RI/RII provokes growth inhibition. However, the relevance of differential modulation of PKA-R subunits during the 8-Cl-cAMP-induced growth inhibition and cytotoxicity is still under debate (18–20). Furthermore, we recently showed that activation of protein kinase C (PKC), rather than PKA, is involved during the 8-Cl-cAMP-induced growth inhibition in mouse fibroblast DT cells (21).

MAPKs are other important candidates that might be involved in the 8-Cl-cAMP-induced growth inhibition and apoptosis. It has long been reported that cAMP and MAPKs are closely related in the regulation of cell proliferation (reviewed in ref. 1). There are distinct subfamilies in MAPKs that have evolved for transmitting different types of signals, such as the extracellular signal-regulated kinase (ERK) for mitogenic stimuli and differentiation and the p38 MAPK and c-Jun NH₂-terminal kinase (JNK) for stress factors (22). p38 MAPK and JNK are usually related to cell growth inhibition or apoptosis, whereas ERK promotes cell proliferation (23), and cAMP signal can intervene during these processes. In many instances, cAMP can inhibit cellular proliferation by blocking ERK signaling stimulated by growth factors (13, 24–26). The activation of p38 MAPK can also be regulated by cAMP signals; for example, cAMP-increasing signals induce p38 MAPK activation and this activation is blocked by PKA catalytic inhibitor in NG108-15 neuroblastoma × glioma hybrid cells (27), SK-N-MC human neuroblastoma cells (28), and Chinese hamster ovary cells stably transfected with the human thyroid-stimulating hormone (TSH) receptor (29). In this study, we tested whether MAPK pathway is involved in the 8-Cl-cAMP-induced growth inhibition and apoptosis and found that p38 MAPK is indispensable in evoking these events.

Materials and Methods

Chemicals and drugs. 8-Cl-cAMP and 8-chloro-adenosine (8-Cl-adenosine) were purchased from Biolog (Bremen, Germany). [γ-32P]ATP (6,000 Ci/mmol) was purchased from Dupont NEN (Boston, MA). Selectide...
HL60 and K562 cells were maintained in RPMI 1640 SB for A/C2 Cancer Res 2005; 65: (11). June 1, 2005 P A P A A B, A Asterisk, j B, subunits was described previously (30). Antibody for phospho-p38 h and A A A Suspended cells were harvested by A/C2 for 10 minutes. Total cell extract (10 Cells were harvested by centrifugation and A for 10 minutes. DNA in the supernatant was purified by phenol/ chloroform extraction and precipitated with ethanol. The DNA was then electrophoresed on 2% agarose gel and visualized by staining with ethidium bromide. Fluorescence-activated cell sorting analysis. Harvested cells were suspended in PBS and then fixed with ice-cold 70% ethanol for at least 3 hours. Fixed cells were stained with 50 µg/mL propidium iodide containing 50 µg/mL RNase A at 37°C for 30 minutes. DNA contents of cells (10,000 cells per experimental group) were analyzed by FACStarPLUS flow cytometer (B&D, Mountain View, CA) using Lysis II and CELL-FIT programs (B&D).

Results

8-Chloro-cyclic AMP–induced growth inhibition is blocked by p38 mitogen-activated protein kinase inhibitor in HL60 cells. Previously, we reported that 8-Cl-cAMP induces inhibition of proliferation and apoptosis in various human cancer cell lines, including SH-SY5Y and HL60 (14). In HL60 (acute promyelocytic leukemia) cells, growth inhibition was induced after the treatment of 8-Cl-cAMP in time- and dose-dependent manners (Fig. 1A and B). However, K562 (chronic myelogenous leukemia) was more resistant to the growth inhibitory effect of 8-Cl-cAMP than HL60. It has been reported that K562 cells express the BCR-ABL fusion protein due to genetic rearrangement that maintains resistance to apoptosis induced under various conditions (31, 32).

To find out the signaling mechanisms involved in this growth inhibition, we first carried out kinase-inhibitor study (Fig. 1C). In parallel with the growth inhibition study in DT cells (21), PKA inhibitor (H89, 10 µmol/L) did not show any recovery effect on the 8-Cl-cAMP-induced growth inhibition, which means that this process is independent of PKA catalytic activity. GF109203x (3 µmol/L), a potent PKC inhibitor, could not reverse the 8-Cl-cAMP-induced growth inhibition, which seems to be incompatible to our previous experiments using DT cells (21). However, unlike the case in DT cells, 8-Cl-cAMP induces not only inhibition of proliferation but also apoptosis in HL60 cells. Therefore, this result can be interpreted that 8-Cl-cAMP may evoke apoptosis via another signaling pathway(s) rather than PKC activation through which inhibition of proliferation is induced.

Figure 1. 8-Cl-cAMP-induced growth inhibition is blocked by p38 MAPK inhibitor. A and B, cell growth test after 8-Cl-cAMP treatment. HL60 and K562 cells were seeded at the concentration of 1 x 10^5 per well on 12-well plates. A, HL60 and K562 cells were treated with 8-Cl-cAMP (10 µmol/L) for the indicated times. B, HL60 and K562 cells were treated with the indicated concentrations of 8-Cl-cAMP for 3 days. After the treatment, cell numbers were measured with Coulter counter and normalized to control groups (100%). K562 cells were more resistant to the growth inhibitory effect of 8-Cl-cAMP than HL60 cells. Asterisks, differences between two groups (P < 0.01, two-tailed t test). C, effects of kinase inhibitors on the 8-Cl-cAMP-induced growth inhibition. HL60 cells were seeded at the concentration of 1 x 10^5 per well on 12-well plates and incubated with 8-Cl-cAMP (8CI, 10 µmol/L) + various kinase inhibitors for 3 days. Only SB203580 (SB) could attenuate the 8-Cl-cAMP-induced growth inhibition. Asterisk, difference from 8-Cl-cAMP alone–treated group (P < 0.01, two-tailed t test). The inhibitors used in this experiment were PD98059 (PD, 10 µmol/L), SP600125 (SP, 10 µmol/L), SB203580 (10 µmol/L), GF109203x (GF; 3 µmol/L), and H89 (10 µmol/L).
Neither PD98059 (10 μmol/L; ERK inhibitor) nor SP600125 (10 μmol/L; JNK inhibitor) could reverse the 8-Cl-cAMP-induced apoptosis. However, SB203580 (10 μmol/L; a p38 MAPK inhibitor) attenuated the growth inhibitory effect when cotreated with 8-Cl-cAMP (Fig. 1C). In this respect, p38 MAPK can be regarded as a signaling mediator of the 8-Cl-cAMP-induced growth inhibition and/or apoptosis in HL60 cells.

8-Chloro-cyclic AMP–induced apoptosis is mediated by p38 mitogen-activated protein kinase activation. 8-Cl-cAMP-treated HL60 cells showed cell body shrinkage, nuclear condensation, and nuclear fragmentation (Fig. 2A, C, and D), which are the typical characteristics of apoptosis (33). As might be expected, this apoptotic morphology disappeared by the inhibition of p38 MAPK activity (Fig. 2A). Moreover, in DNA fragmentation assay and fluorescence-activated cell sorting analysis, p38 MAPK inhibitor completely restored the 8-Cl-cAMP-induced apoptosis (Fig. 2C and D). However, 8-Cl-cAMP-treated K562 cells did not show any morphologic differences from mock-treated control cells (Fig. 2B).

To investigate whether p38 MAPK activation is actually involved in the 8-Cl-cAMP-induced apoptosis, we measured the phosphorylated (activated) form of p38 MAPK using a phospho-specific p38 MAPK antibody. In HL60 cells, phosphorylated p38 MAPK started to increase at 24 hours after the treatment of 8-Cl-cAMP (Fig. 3A), and this phosphorylation exhibited dosage dependency (Fig. 3C). However, K562 cells, which are more tolerant to 8-Cl-cAMP, showed constant level of phospho–p38 MAPK (Fig. 3D). Furthermore, MKK3/6, which are known as upstream kinases of p38 MAPK, were also activated by the treatment of 8-Cl-cAMP in HL60 cells (Fig. 3D). All these data suggest that the 8-Cl-cAMP-induced apoptosis is mediated by MKK3/6-p38 MAPK signaling pathway.

p38 mitogen-activated protein kinase activation is upstream of protein kinase C activation during the 8-chloro-cyclic AMP–induced apoptosis. Although PKC inhibitor could not attenuate growth inhibition by 8-Cl-cAMP in HL60 cells, it is necessary to clarify the hierarchical relationship between p38 MAPK and PKC during the 8-Cl-cAMP-induced growth inhibition. To investigate whether PKC activity is also increased by 8-Cl-cAMP in HL60 cells, we carried out PKC activity assay using a PKC-specific peptide substrate, Selectide. Although the 8-Cl-cAMP-induced apoptosis was not attenuated by the cotreatment of PKC inhibitor (Fig. 1C), 8-Cl-cAMP was still able to stimulate the PKC activity in HL60 cells (Fig. 4A). It can be argued that induction of apoptosis, which is independent of PKC activation, might be more predominant than inhibition of proliferation in HL60 cells that total cell number decreased by 8-Cl-cAMP was hardly affected by PKC inhibition.

After the treatment of 8-Cl-cAMP, both p38 MAPK and PKC were activated as shown previously. Irrespective of the presence of PKC inhibitor (GF109203x), 8-Cl-cAMP still increased the phosphorylation of p38 MAPK (Fig. 4B). On the contrary, PKC activity was reduced to the normal level by the cotreatment of p38 MAPK inhibitor (SB203580, Fig. 4C). Practically, both SB203580 and GF109203x could recover DT cell growth suppressed by 8-Cl-cAMP (Fig. 4D). These results suggest that after 8-Cl-cAMP treatment PKC activation also occurs in HL60 cells, and this PKC activation is regulated by p38 MAPK activation (i.e., PKC resides or acts downstream of p38 MAPK).

p38 mitogen-activated protein kinase activation by 8-chloro-cyclic AMP is independent of PKA catalytic activity. It has been reported that p38 MAPK activation is controlled by PKA under certain conditions (27–29). For example, in Chinese hamster ovary cells transfected with TSH receptor, TSH and forskolin induced MKK3/6 and p38 MAPK activation, and this activation was blocked by PKA inhibitor, H89 (29). To verify whether p38 MAPK activation by 8-Cl-cAMP is also dependent on PKA activity, we did phospho–p38 MAPK Western blotting after cotreatment of 8-Cl-cAMP and H89 (10 μmol/L). H89 has been known to completely block PKA enzymatic activity even at 5 μmol/L (14). Nevertheless, it had no effect on p38 MAPK phosphorylation induced by 8-Cl-cAMP (Fig. 4E). The type I PKA regulatory subunit α (Rlo) down-regulation by 8-Cl-cAMP, which has been regarded as an important cause of the 8-Cl-cAMP-induced growth inhibition (17), was also returned to normal levels by blocking the p38 MAPK activation (Fig. 4F). Therefore, p38 MAPK activation is not regulated by PKA at least in our experimental system. On the contrary, it suggests that Rlo down-regulation signal by 8-Cl-cAMP may be transduced through the p38 MAPK pathway.

8-Chloro-cyclic AMP–induced apoptosis is blocked by p38 MAPK inhibition. A, acridine orange staining in HL60 cells. HL60 cells were treated with 8-Cl-cAMP (10 μmol/L) in the presence or absence of SB203580 (10 μmol/L) for 3 days. To show the nuclear morphology, HL60 cells were stained with acridine orange (10 μg/mL) and then observed under a fluorescence microscope. 8-Cl-cAMP-treated cells showed nuclear fragmentation and condensation (arrows), which was blocked by the cotreatment of SB203580 (10 μmol/L). B, acridine orange staining in K562 cells. K562 cells were also treated as in (A), and acridine orange staining was done. 8-Cl-cAMP could not affect the cell morphology. C, DNA fragmentation assay. HL60 cells were treated with 8-Cl-cAMP (10 μmol/L) in the presence or absence of SB203580 (10 μmol/L) for 3 days. Then, chromosomal DNA was prepared and electrophoresed in 2% agarose gel. Chromosomal DNA obtained from 8-Cl-cAMP-treated cells showed fragmented pattern, which was disappeared by p38 MAPK inhibition. D, fluorescence-activated cell sorting analysis of cell cycle. HL60 cells were treated with 8-Cl-cAMP (10 μmol/L) in the presence or absence of SB203580 (10 μmol/L) for 3 days and then stained with 10 μg/mL propidium iodide. After propidium iodide staining, the cell cycle profile was measured using flow cytometry. Numbers, percentage of sub-G1 phase (i.e., apoptotic population). 8-Cl-cAMP increased sub-G1 population (27.4%), which was diminished by the treatment with 10 μmol/L SB203580 (8.5%).
8-Cl-cAMP induction of apoptosis via p38 MAPK

p38 mitogen-activated protein kinase activation by 8-chloro-cyclic AMP is also mediated by its metabolite, 8-chloro-adenosine. As we have shown previously (21), metabolism of 8-Cl-cAMP into 8-Cl-adenosine or further metabolites is indispensable for the induction of growth inhibition and cell death in various cancer cell lines. In HL60 cells, the 8-Cl-cAMP-induced or 8-Cl-adenosine-induced growth inhibition is also blocked by the cotreatment of an adenosine kinase inhibitor, A134974 (10 μmol/L; Fig. 5A). Furthermore, A134974 down-regulated the 8-Cl-cAMP-induced or 8-Cl-adenosine-induced apoptosis, which was assessed by poly(ADP-ribose) polymerase (PARP) cleavage (Fig. 5B). To clarify whether p38 MAPK activation is also dependent on the 8-Cl-cAMP metabolism, we checked cell growth after 8-Cl-adenosine treatment in the presence or absence of 10 μmol/L SB203580. Just as with 8-Cl-cAMP, 8-Cl-adenosine did not induce growth inhibition in the presence of SB203580 (Fig. 5C). We also looked at the p38 MAPK activation by Western blotting after 8-Cl-adenosine treatment (Fig. 5D). Both 8-Cl-cAMP and 8-Cl-adenosine increased the p38 MAPK phosphorylation, and it returned to normal levels by the cotreatment of A134974. We can surmise from these results that the 8-Cl-cAMP-induced p38 MAPK activation requires the metabolic modification to 8-Cl-adenosine by adenosine kinase.

Figure 3. p38 MAPK is activated after 8-Cl-cAMP treatment. A and B, phopho-p38 Western blotting after 8-Cl-cAMP treatment. HL60 (A) and K562 (B) cells were treated with 8-Cl-cAMP (10 μmol/L) for the indicated times; then, Western blotting was carried out using anti-phospho-p38 (pp38) or anti-total p38 (p38) antibody. Phospho-p38 increased time dependently in HL60 after 8-Cl-cAMP treatment, whereas it was not changed in K562 cells. Total p38 showed constant level in both cell lines. C, phopho-p38 Western blotting in HL60 cells. After treatment of indicated concentrations of 8-Cl-cAMP for 3 days, phopho-p38 Western blotting was carried out. Even at 2 μmol/L 8-Cl-cAMP, p38 MAPK was phosphorylated eminently. D, phoshpo-MKK3/6 Western blotting. After 8-Cl-cAMP was treated as in (A), phosphorylation (activation) of MKK3/6 was determined by Western blotting using anti-phospho-MKK3/6 (pMKK3/6) antibody in HL60 cells. pMKK3/6 was also increased after 8-Cl-cAMP treatment.

Figure 4. p38 activation is upstream of PKC activation and is independent of PKA activation. A, PKC activity assay. After 8-Cl-cAMP treatment to HL60 cells for indicated times, PKC activity was evaluated using a PKC-specific substrate, Selectide, as mentioned in Materials and Methods. After 48 hours, PKC enzymatic activity started to increase. Asterisks, differences from 0-hour group (P < 0.01, two-tailed t test). B, effect of PKC inhibitor on p38 phosphorylation by 8-Cl-cAMP. HL60 cells were treated with 8-Cl-cAMP (10 μmol/L) in the presence or absence of GF109203x (3 μmol/L) for 3 days; then, phopho-p38 Western blotting was carried out. GF109203x could not attenuate p38 phosphorylation induced by 8-Cl-cAMP. C, effect of p38 inhibitor on PKC activation by 8-Cl-cAMP. HL60 cells were treated with 8-Cl-cAMP + GF109203x (3 μmol/L) or SB203580 (10 μmol/L) for 3 days; then, PKC activity was measured using Selectide peptide as a substrate. SB203580 completely reduced PKC activity increased by 8-Cl-cAMP. D, effect of p38 and PKC inhibitors on the 8-Cl-cAMP-induced growth inhibition in DT cells. 8-Cl-cAMP (10 μmol/L) was treated to mouse fibroblast DT cells with p38 or PKC inhibitors for 3 days; then, cell numbers were counted. Both SB203580 (10 μmol/L) and GF109203x (3 μmol/L) could recover cell growth inhibited by 8-Cl-cAMP. Asterisks, differences from 8-Cl-cAMP group (P < 0.01, two-tailed t test). E, effect of PKA inhibitor on p38 phosphorylation by 8-Cl-cAMP. HL60 cells were treated with 8-Cl-cAMP in the presence or absence of H89 (10 μmol/L) for 3 days; then, phopho-p38 level was measured by Western blot analysis. H89 could not attenuate p38 phosphorylation induced by 8-Cl-cAMP. F, effect of p38 inhibitor on Ria down-regulation induced by 8-Cl-cAMP. HL60 cells were treated with 8-Cl-cAMP in the presence or absence of SB203580 (10 μmol/L); then, Ria level was measured. SB203580 could reverse the Ria protein level decreased by 8-Cl-cAMP.
A/2

Discussion

Due to its growth inhibitory effect, 8-Cl-cAMP has been studied as one of the candidates for anticancer therapeutic agent for many years (15, 16). 8-Cl-cAMP inhibits cell proliferation and induces cell death (apoptosis) in various cell lines. In general, this growth inhibition occurs more evidently in transformed cell lines than in normal cell lines. For example, 8-Cl-cAMP induced growth inhibition and apoptosis in MCF-7 human breast cancer cells, but it hardly affected the growth of MCF-10A human mammary epithelial cells (14). However, the mechanisms of its anticancer activity have not been fully understood and are still under debates. Cho-Chung et al. have done considerable amount of studies that suggest 8-Cl-cAMP induces growth inhibition by the differential regulation of PKA regulatory subunits, RI and RII (15, 17, 34, 35); that is, 8-Cl-cAMP decreases the level of RI protein that distorts the intracellular RI/RII ratio, which is important for the maintenance of cellular physiology and the effects of 8-Cl-cAMP can be mimicked by the addition of RIo antisense oligonucleotide.

On the contrary, several lines of evidence indicate that the regulation of PKA-R subunits by 8-Cl-cAMP is not the major cause of growth inhibition (18–20, 36). 8-Cl-cAMP could not induce growth inhibition in the presence of IBMX (a phosphodiesterase inhibitor) and A134974 (an adenosine kinase inhibitor), which lead to the blockage of 8-Cl-cAMP metabolic pathway (20). In addition, not 8-Cl-cAMP, but its metabolite, 8-Cl-adenosine, is the key molecule that is responsible for the growth inhibitory effect. Furthermore, during this growth inhibitory process, PKA catalytic activity is not required. Instead, as in our previous report, it was found that PKC activation was involved in the 8-Cl-cAMP-induced growth inhibition in DT cells (21).

In HL60 cells, PKC activity was also increased by 8-Cl-cAMP treatment, but this activation was not essential in inducing the growth inhibition or apoptosis. If 8-Cl-cAMP induces proliferation inhibition and apoptosis through different signaling pathways, in HL60 cells where the 8-Cl-cAMP-induced apoptosis occurs more dominantly, the contribution of PKC activation to the total cell growth may not be so critical. In this report, we found that 8-Cl-cAMP induces apoptosis through another signaling pathway, p38 MAPK activation.

p38 MAPK activation is well known to mediate apoptotic signal transduction. Inhibition of p38 MAPK pathway blocked apoptosis induced by various stimuli, such as trophic factor deprivation (37–39), oxidative stresses (40, 41), ceramides (42, 43), and UV radiation (44–46). In addition, there are many experimental evidence that imply cross-talk between p38 MAPK and cAMP signaling. For example, TSH and forskolin induced p38 MAPK activation in thyroid cells or Chinese hamster ovary cells stably transfected with TSH receptor (29). According to the results presented here, the 8-Cl-cAMP-induced apoptosis was also mediated by p38 MAPK activation in HL60 cells. However, in K562 cells, which are tolerable to many apoptosis-inducing agents, p38 MAPK was not activated and apoptotic cell death was not observed after 8-Cl-cAMP treatment. The mechanisms by which the BCR-ABL-positive leukemia cells resist the apoptotic signals are not fully understood, although delayed caspase activation or increased Bel-XL level in K562 cells has been reported (32). We showed here that p38 MAPK activation can be regarded as another determinant to execute apoptosis in leukemia cells.

In this study, it was also found that the 8-Cl-cAMP-induced apoptosis is dependent on its metabolic pathway because it is entirely blocked by an adenosine kinase inhibitor. 8-Cl-cAMP can be converted to 8-Cl-adenosine by the actions of phosphodiesterase and nucleotide phosphatase, and 8-Cl-adenosine is further metabolized by adenosine kinase into 8-Cl-AMP or 8-Cl-ATP. In
many experimental systems, 8-Cl-cAMP must be metabolized to induce growth inhibition; nevertheless, it is still debatable (18–20, 36). It cannot be overlooked that down-regulation of PKA-RI subunit by 8-Cl-cAMP is a critical aspect during the induction of growth inhibition. However, 8-Cl-adenosine can also decrease the Rls subunit, which is blocked by adenosine kinase inhibitor (data not shown). Additionally, p38 MAPK pathway may be involved in this Rls down-regulation process because cotreatment of the p38 MAPK inhibitor, SB203580, reinstated the Rls protein level decreased by 8-Cl-cAMP and 8-Cl-adenosine. Therefore, two hypotheses explain the growth inhibition mechanism of 8-Cl-cAMP; that is, Rls down-regulation by 8-Cl-cAMP itself or the influence of its metabolic pathway do not seem to be so incompatible. To elucidate the exact mechanism of the 8-Cl-cAMP-induced growth inhibition or apoptosis, further researches must be carried out more delicately.

References
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