A dissertation for the Degree of Master

Phosphodiesterase 4D Inhibition Induces Cell Cycle Arrest and Neural Differentiation via cAMP/PKA Signaling in Primary Glioblastoma Multiforme

초대다형성교아종에서의 cAMP/PKA 신호경로를 통한 세포주기억제와 신경분화를 유도시키는 인산디에스테라아제-4 아형 D 억제제에 관한 연구

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ABSTRACT

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Glioblastoma Multiforme (GBM) is a most common malignant brain tumor in adult with a survival of less than a year due to its resistances. Recent studies have been reported that glioblastoma initiated from CD133 positive subpopulation, known as cancer stem cell population, which takes very small portion in glioblastoma. Although it has been regarded cyclic AMP phosphodiesterase-4 (PDE4) inhibitor as a potent anti-tumor agent, it is still remained unclear how PDE4 inhibition regulates anti-cancer effect in GBM.

Thus, in this study, we investigated the novel phosphodiesterase-4 subtype D (PDE4D) inhibitor CG500354 for GBM and determined its ability and cellular signaling pathway in GBM. We found that CG500354 remarkably induced GBM growth arrest by down-regulating cyclin B1 while increasing p21 and p27 mRNA expression. In immunocytochemistry, the expression of Nestin, neural progenitor marker, was decreased in CG500354 treated GBM cells while the expression of GFAP, astrocyte marker, and Tuj-1, neuronal marker, were significantly increased. Regarding PDE4D is involved in cAMP/PKA signaling pathway, we determined the protein levels of phosphorylated protein kinase A (p-PKA) and phosphorylated CREB (p-CREB). In Western blot analysis, CG500354 increased the protein levels of GFAP and Tuj-1 as a result shown in immunocytochemistry. Moreover, CG500354 significantly increased the phosphorylation of PKA and CREB. These results were also confirmed and supported by cAMP measurement assay. Besides, CG500354 increased the p53 expressing GBM cells in immunocytochemistry. Taken together, CG500354 takes important roles in GBM growth arrest regulation through up-regulating the levels of cell-cycle-related and tumor-suppressor markers as well as neural differentiation markers.

Keywords : Glioblastoma, PDE4D inhibitor, cAMP, neural differentiation, anti-cancer

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LIST OF ABBREVIATION

GBM	Glioblastoma multiform
CSLC	Cancer stem-like cell
PDE4	Phosphodiesterase-4
PDE4D	Phosphodiesterase-4 subtype D
cAMP	Cyclic adenosine monophosphate
CREB	cAMP response element-binding
PKA	Protein kinase A
GFAP	Glial fibrillary acidic protein
Tuj-1	Neuron Class III β-tubulin
PLO	Poly-ornithine
FN	Fibronectin
bFGF	Basic fibroblast growth factor
EGF	Epidermal growth factor
RT-PCR	Reverse transcription-polymerase chain reaction

TABLE OF CONTENTS

INTRODUCTION

GBM is the most common lethal primary brain tumor in adult with a Median survival of less than 12 months due to its radioresistant (Bao et al., 2006) and chemoresistant (Liu et al., 2006; Stupp et al., 2009). Recent studies have been suggested that cancer stem-like cell (CSLC) population can be targeted for GBM therapy (Lathia et al., 2010). And also reported that GBM subpopulation expresses CD133 and is enriched for CSLCs (Bao et al., 2006; Collins et al., 2005; Eramo et al., 2008; Singh and Dirks, 2007). This subpopulation show an increased tumorigenic potential than populations that do not express CD133 (Beier et al., 2007; Galli et al., 2004; Reya et al., 2001; Yuan et al., 2004).

There were many targeted therapies of tumorigenic cell populations, but they have achieved not meaningful effects yet (Yang et al., 2007). Several studies have investigated cyclic AMP (cAMP) phosphodiesterase-4 (PDE4) can play a role in a mechanism of tumor suppression (Goldhoff et al., 2008; Moreno et al., 2006; Yang et al., 2007) Many genes related with cAMP signaling pathway can act as oncogenes or oncogene suppressors (Kirschner et al., 2000; Landis et al., 1989; Lania et al., 2001; Lucchi et al., 2011; Persani et al., 2000). In addition, the phosphorylation of CREB by PKA-mediated cAMP-dependent signaling cascade appears to play a role in differentiation (Impey et al., 2004; Sato et al., 2006). Rolipram, a selective PDE4 inhibitor, is commonly known as a memory enhancer (Otmakhov et al., 2004; Romano et al., 1996) by increasing cAMP and phosphorylation of CREB (Park et al., 2012; Schneider, 1984). Recently, Rolipram has

been used for glioblastoma study because of its role in cAMP signaling pathway (Li et al., 2011). Also Forskolin is already known for cAMP regulator (Farias et al., 2008). Thus, we treated these two known chemicals to prove our new finding chemical CG500354 and its targeting specific mechanism. There are four PDE4 subfamilies A to D which have more than 20 different isoforms (Li et al., 2011) and regulate cAMP through their hydrolytic activity (Houslay and Adams, 2003; Marko et al., 2000).

Currently from our work, suggested that new finding chemical CG500354, PDE4D inhibitor, might be the novel regulator targeting GBM by regulating cAMP/PKA signaling pathway which takes important role in growth, differentiation, gene regulation and apoptosis (Walsh and Van Patten, 1994). In this study, we investigate that CG500354 induces growth arrest of GBM by up-regulating of p21, p27 and p53 and force to differentiate into neural subtypes. This neural differentiation is mediated via cAMP/PKA signaling pathway activated by CG500354. Finally, these results of CG500354 reflect that of both well-known tumor suppressor Rolipram and Forskolin.

MATERIALS AND METHODS

Isolation of Primary GBM Cells

Following informed consent and in accordance with the appropriate Institutional Review Boards, GBM specimen 559 (GBM559) was obtained from a patient undergoing surgery at the Samsung Medical Center. The tumor sample was classified as GBM on the basis of World Health Organization. Within an hour of surgical resection, the tumor was mechanically and enzymatically dissociated into single cells GBM559 was briefly maintained in NBE neurosphere culture medium: Neurobasal-A medium (Invitrogen, Carlsbad, CA, USA) supplemented with 2mM glutamine (Invitrogen), 100 units per ml penicillin, 100 mg/ml streptomycin (Invitrogen), recombinant human basic fibroblast and epidermal growth factors (50 ng/ml each; Invitrogen), N2 and B27 supplements (Invitrogen).

Chemicals

CG500354 (Patent No. 61/470884) was provided from CrystalGenomics, Inc., republic of Korea. Rolipram ($C_{16}H_{21}NO_3$; 4-[3-(Cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone) and Forskolin ($C_{22}H_{34}O_7$; 7β-Acetoxy-8,13-epoxy-1α,6β,9α-trihydroxylabd-14-en-11-one) were purchased from Sigma Aldrich, USA

siRNA inhibition study

To specifically inhibit PDE4D, siRNA knockdown studies were performed using commercial siRNA targeting PDE4D (Dharmacon, ON Target plus SMART pool) along with a non-targeting siRNA (Dharmacon, ON Target plus SMART pool, Cat# D-001810-01, Lafayette, CO, USA). The siRNA transfections were performed according to the manufacturer's instructions. Briefly, the cells were seeded at a concentration of 5 x 10^5 per 6-well plate, and siRNA-containing media (without added antibiotics) were added when the cells reached 60% confluence. The cells were incubated with 50 nM siRNA for 24 hr to evaluate mRNA expression and for 48 hr to evaluate protein expression. After these incubations, RNA and protein extractions were performed for western blot and RT-PCR analyses.

Western blot analysis

Cell were lysated with PRO-PREP™ Protein Extraction Solution containing 1mM phenylmethylsulfonyl fluoride; PMSF, 1mM ethylendiamine tetraacetic acid;EDTA, 1μM Pepstatin A, 1μM Leupeptin, 1μM Aprotinin. Protein concentrations were determined using the DC assay kit (Bio-Rad, Hercules, CA, USA), Protein samples were separated on 10–15% SDS-PAGE and then transferred to nitrocellulose membranes at 50 V, 350 mA for 3 hours. All antibodies were used according to the manufacturer's instructions. Antibodies used for Western blot analysis were mouse monoclonal anti-GFAP (ab4648; ABCAM), mouse monoclonal anti-Tuj1 (MMS435; Covance), mouse monoclonal anti-P53 (2524; Cell Signaling Technology Inc.), rabbit monoclonal anti-p-CREB (9198; Cell

Signaling Technology Inc.), rabbit monoclonal anti-p-PKA (5661; Cell Signaling Technology Inc.), mouse monoclonal anti-Gapdh (MAB374; Millipore) and images were taken using FluorChem HD2 (Alpha Innotech)

Immunocytochemistry

Cells were grown on PLO and FN coated coverslips ($2x10^4$/slide). The cells were fixed with 4% paraformaldehyde for 15 min at RT and permeabilized with PBS containing 0.25% Triton X-100 for 10 min. The cells were then incubated with 5% normal goat serum (Zymed, San Francisco, CA, USA) for an hour and then incubated with primary antibodies for overnight at 4℃ and then incubated with appropriate corresponding secondary antibodies conjugated with fluorescent dye (1:200) for an hour and DAPI staining for 10min. Antibodies used for immunocytochemistry were mouse monoclonal anti-Nestin (MAB5326; Millipore, Billerica, MA, USA), mouse monoclonal anti-GFAP (ab4648; ABCAM), mouse monoclonal anti-Tuj1 (MAB1195; R&D systems) mouse monoclonal anti-P53 (2524; Cell Signaling Technology Inc.). The secondary fluorescence conjugated antibodies, goat anti-mouse Alexa Fluor 488, and goat anti-rabbit Alexa Fluor 488 (Invitrogen), were each used at a 1: 1000 dilution. DAPI (vector laboratory) was used for counterstaining. Coverslips were mounted with florescent mounting solution (DAKO) and observed under confocal microscopy system.

cAMP measurement assay

GBM cells were treated with DMSO and CG500354 (3 uM/ml) for 72h. cAMP level

was measured from collected supernatants using cyclic AMP EIA Kit (Cayman chemical company, Ann Arbor, MI) according to manufacturer's instruction. cAMP values were calculated by multifunction reader (Tecan, San Jose, CA)

RT-PCR and real-time PCR.

Total RNA was isolated from cells using TRIzol reagent following the protocol provided by the manufacturer (Invitrogen). Total RNA was used for cDNA synthesis using Maxime RT Premix (Intron, Republic of Korea). For RT-PCR, cDNA and primers were combined with a PCR premix (Bioneer, Republic of Korea). Real-time PCR was performed by mixing cDNA with primers, and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). Real-time PCR was performed using an ABI 7500 Real-time PCR System with supplied software (Applied Biosystems), according to the manufacturer's instructions. RNA expression levels were compared after normalization to endogenous GAPDH.

Flow cytometry analysis

GBM cells were treated with DMSO and CG500354 for 72h and stained with anti-CD133/2-PE antibody (Miltenyi Biotec, Bergisch Gladbach, Germany) for 30 min, CD133 stained GBM cells were analyzed by flow cytometry FACSCalibur (Becton Dickinson, San Jose, CA).

Statistical analysis

All experiments were conducted at least in triplicate (n = 3), and the results are expressed as the mean ± SD. The statistical analyses were conducted using an analysis of variance (ANOVA) followed by Duncan's multiple range tests or Student's t-test. A value of $P < 0.05$ was considered significant (*, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$).

RESULTS

PDE4D inhibitor induces growth arrest of GBM neurospheres without programmed cell death.

The relationship between tumor-suppression and neural differentiation of GBM cells were already been reported but tumor-suppression by PDE4D inhibitor have rarely been studied. To identify this relationship, we treated CG500354 to GBM-derived neurosphere cells for 72 hours and assessed not only decrease of growth rate but also increase of neural differentiation potential. Human derived GBM cells usually grow up as a sphere form in a non-adherent culture condition. To identify CG500354 potential, we cultured GBM cells on Poly-ornithine- and Fibronectin-(PLO/FN) coated dish for adherent culture condition. CG500354 treated GBM neurosphere cells showed morphological change which indicates increase of differentiation potential (Fig. 1A). As additional evidences, we performed cell cycle assay using quantitative RT-PCR analysis and the result revealed a remarkable down-regulation in cyclin B1, the cell-cycle arrest-related gene, and two-fold up-regulation in p21 and p27, the cyclin-dependent kinase inhibitor. (Fig. 1B) To analyze whether growth arrested cells in CG500354 treated GBM cell population undergo the apoptotic pathway by its cytotoxicity, we assessed the cell viability test in both DMSO and CG500354 treated cell populations. The viability of living cells in both populations marked 86% and 79%, respectively (Fig. 1C). Thus, CG500354 indicated to arrest the growth of GBM cells without activating the apoptotic pathway.

PDE4D inhibitor induces cell differentiation and attenuates the self-renewal potential.

To define the relationship of CG500354 with neural differentiation, we seeded cells on PLO/FN coated slides (2 x 10^4 cells per well of 24-well plate) and treated CG500354 in concentration of 1uM, 3uM and 10uM for 72 hours. Subsequently, cells were stained by immunocytochemistry with neural progenitor marker Nestin and neural differentiation markers GFAP as well as Tuj-1 (Fig. 2A). We observed that CG500354 induced changes of neural cell marker expression in a dose-dependent manner. We found that concentration of 3uM was most efficient. The percent of Nestin-expressing cells in concentration of 3uM was decreased 73.51%. In opposition, GFAP- and Tuj-1-expressing cells were increased 19.2% and 63.32%, respectively (Fig. 2B). Consistent with previous immunocytochemistry data showing that CG500354 induces neural differentiation through the expression of GFAP and Tuj-1, protein expressing was also significantly up-regulated in CG500354 treated GBM cells (Fig. 3A and B). Analog to neural differentiation markers, the expression of phosphorylation p53 was up-regulated following CG500354 treatment. These observations led us to pursue PDE4D as a novel target.

CG500354 mimic si-PDE4D, Rolipram and Forskolin role in GBM cells.

Next, we investigated the function of CG500354 in GBM cells by comparing with siRNA and mimetic substances. In si-PDE4D transfected GBM cells, the number of Nestin-expressing cells was diminished 48.5%, whereas both GFAP- and Tuj-1expressing cells were increased 24.8% and 41%, respectively (Fig. 4A). Homolog, GFAP and Tuj-1 protein levels were significantly higher than si-Negative (Fig. 5A). Both immunocytochemistry and western blot analysis revealed that si-PDE4D regulated the expression of neural differentiation markers as CG500354. As known that Rolipram inhibits PDE4 and Foskolin induces cAMP/PKA signaling pathway, we treated GBM cells with both mimetic substances. Like CG500354, treatments of Rolipram and Foskolin down-regulated the Nestin expression level and simultaneously up-regulated GFAP and Tuj-1 expression level (Fig. 4B and 5B). Both mimetic substances showed identical phenomena as CG500354 and together with si-PDE4D provided direct evidence that CG500354 work as a multi-controller which inhibit PDE4 and activate the cAMP signaling pathway.

CG500354 induces neural differentiation via regulating cAMP/PKA signaling pathway

cAMP/PKA signaling pathway is well-known for its differentiation potential. To verify the PDE4D related mechanism, we performed western blot analysis using downstream

targets, namely PKA and CREB. Treatments of CG500354 induced phosphorylation of PKA and CREB in dose-dependent manner. Therefore, GBM cells led to differentiate by cAMP/PKA signaling pathway activation (Fig. 6A). To compare the regulation of end target CREB in CG500354 and mimetic substances, we treated Forskolin which showed approximately 11-fold up-regulation of protein level and Rolipram which also induced significant up-regulation (Fig. 6B and C). Next, we analyzed the level of cAMP which expected as promoter for the cAMP/PKA signaling pathway. After CG500354 treatment, we harvested the supernatants from the cell culture and measured the secreted cAMP concentration by Cyclic AMP EIA Kit. The result revealed about 4.5-fold higher cAMP secretion level in CG500354 treated GBM cells (Fig. 7). These data provided that cAMP and its related downstream protein PKA and CREB were regulated by CG500354, systemically.

Following GBM neural differentiation, CG500354 increased p53-expressing cell population

To explore the growth arrest potential of CG500354, we immunocytochemically stained GBM cells treated CG500354 in various concentrations and mimetic substances (Fig. 8A-C). Homolog to previous data from Fig. 3A showed p53 protein was highly expressed by PDE4D inhibition, the number of p53-expressing cells in comparing to non-treated GBM cells was increased after treatments of CG500354, Rolipram and Forskolin, namely 42.35%, 49.39% and 47.22%, respectively (Fig. 8D). Together with previous data,

these results reflect that CG500354 can play a similar role as known tumor-suppressor Rolipram and Forskolin in GBM cell populations through up-regulating of p53. Therefore, the novel target for PDE4D inhibition might provide better understanding to develop new drugs for GBM in near future.

CD133+ subpopulation in GBM cells were down-regulated by PDE4D inhibitor.

For the last, we investigated effect of CG500354 on the subpopulation of GBM which is commonly known as CSLC niche. This part takes an important role for proliferation and survival in GBM and this can be detected by CD133 marker. Thus, we stained GBM cells with CD133 to classify whether it has CSLC subpopulation or not. We found that there was a CD133-expressing subpopulation. Moreover, we observed that CG500354 targets CD133 positive population in GBM cells (Fig. 9A-C). These results support our main strategy that CG500354 not only accelerate the neural differentiation potential but also makes GBM cells to loss their cancer stemness.

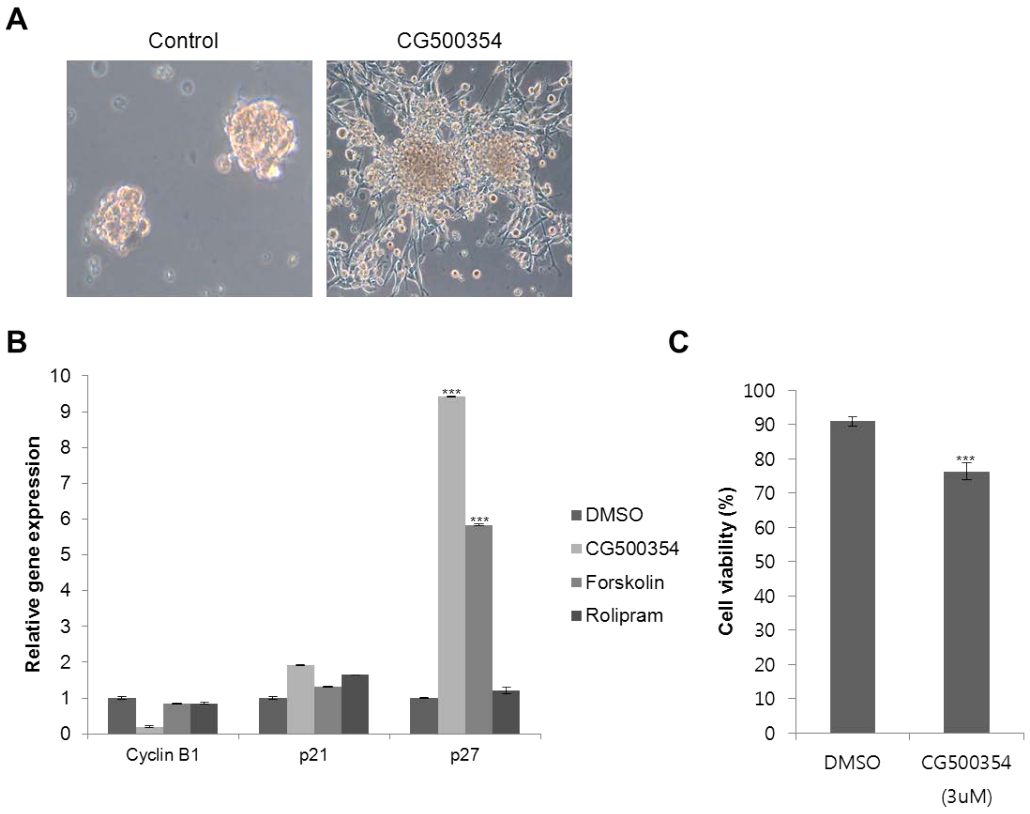

Figure 1. CG500354 induces growth arrest of GBM neurospheres

A) Both phase contrast pictures show morphological change after 72 hours of CG500354 (3 uM) treatment. B) Relative gene expressions of cell cycle-related cyclin B1, p21 and p27 in response to CG500354 treatment were analyzed by quantitative RT–PCR.

C) Living cells were counted and the viability was calculated out of total cell number after 72 hours of CG500354 (3 uM) treatment. Vehicle (=DMSO) was used for control.

*, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

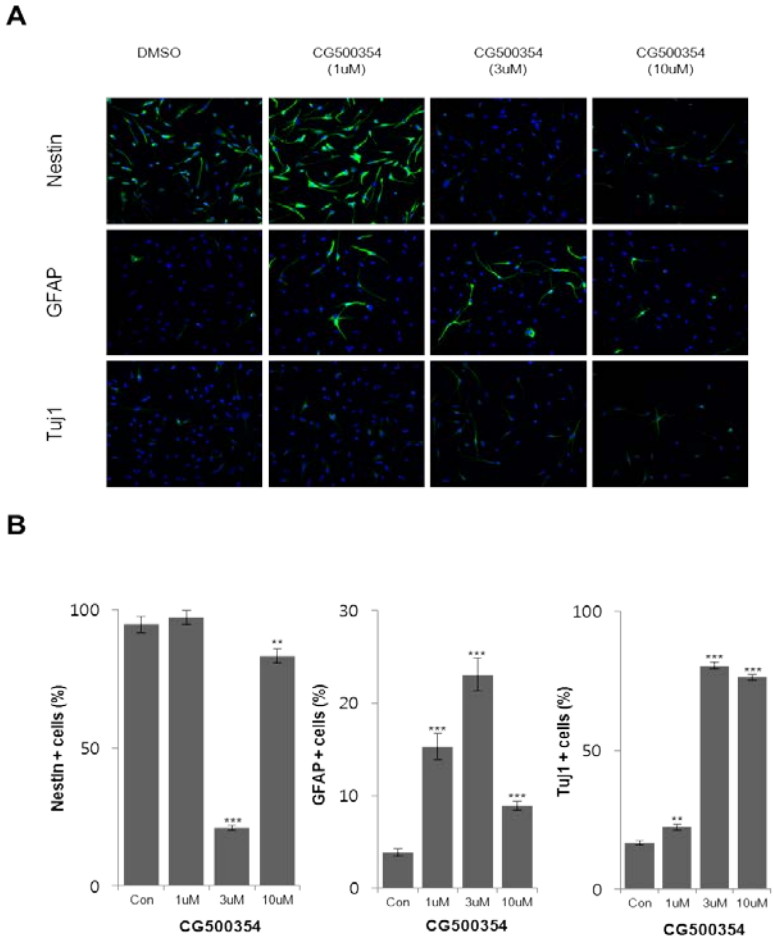

Figure 2. CG500354 induces neural differentiation of GBM neurospheres

A) GBM cells were treated with three different concentrations of CG500354 and stained using immunocytochemistry with anti-GFAP, anti-Tuj1 and anti-Nestin (green). Nuclei counterstained in blue with DAPI. B) The efficiency of neural differentiation of GBM cells was calculated. These graphs indicate percent of Nestin+, GFAP+ and Tuj1+ cells in three different concentrations of CG500354 Vehicle (=DMSO) was used for control. con=Control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

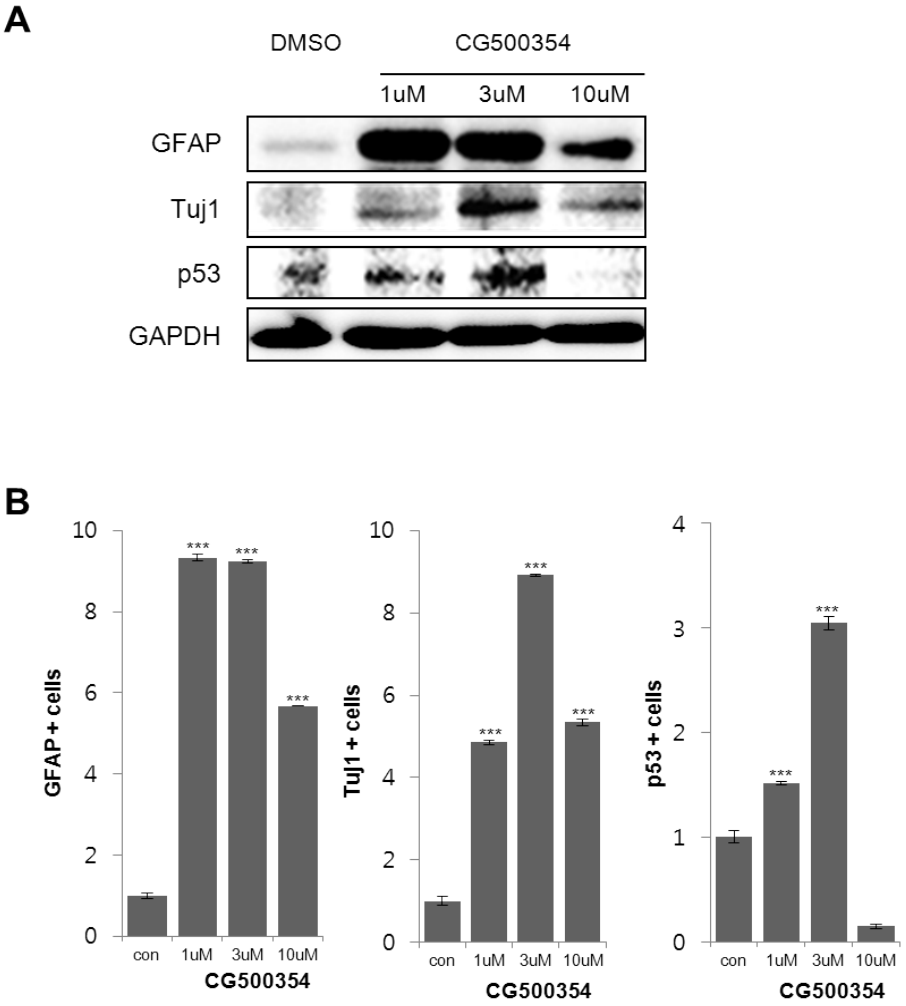

Figure 3. CG500354 induces neural differentiation of GBM neurospheres

A) Western blot analysis show increased level of GFAP, Tuj1 and p-53 after CG500354 treatment. B) The expression of proteins were measured using Image J. Vehicle (=DMSO) was used for control. con=Control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

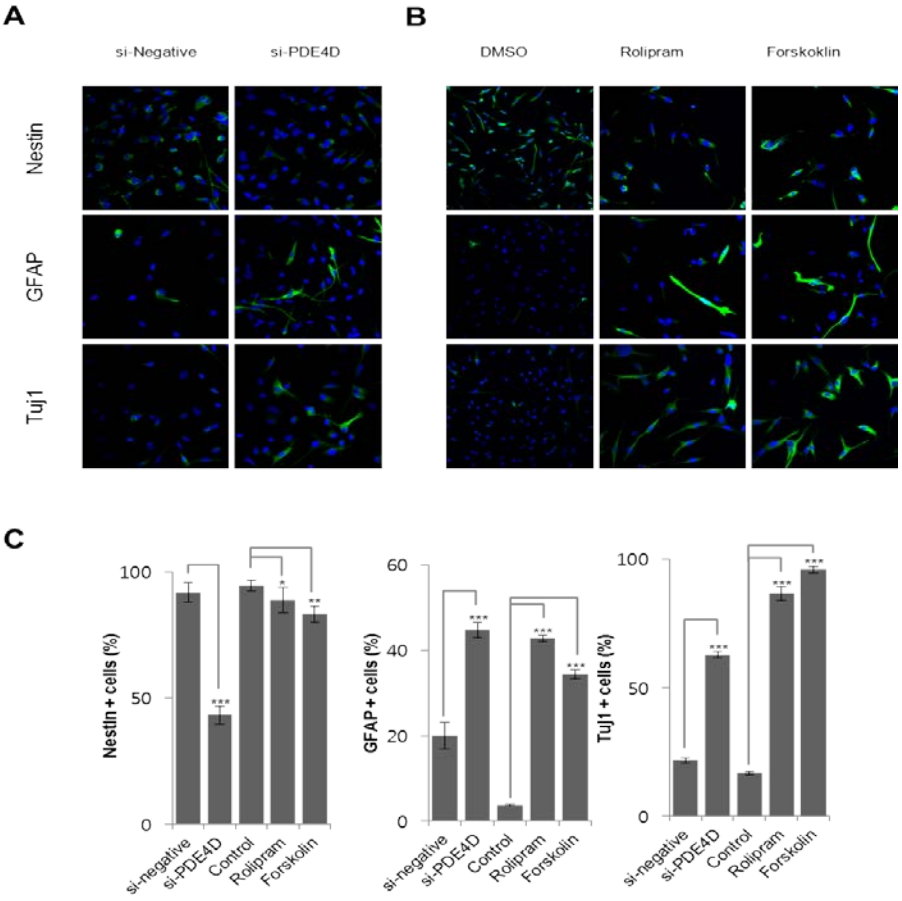

Figure 4. Si-PDE4D, Forskolin and Rolipram induce neural differentiation of GBM neurospheres

A,B) GBM cells were treated with si- PDE4D, Forskolin(10uM) and Rolipram(10uM) and immunostained with anti-GFAP, anti-Tuj1 and anti-Nestin. Nuclei counterstained in blue with DAPI. C) The efficiency of neural differentiation of GBM cells was calculated. These graphs indicate percent of Nestin+, GFAP+ and Tuj1+ cells. Vehicle (=DMSO) was used for control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

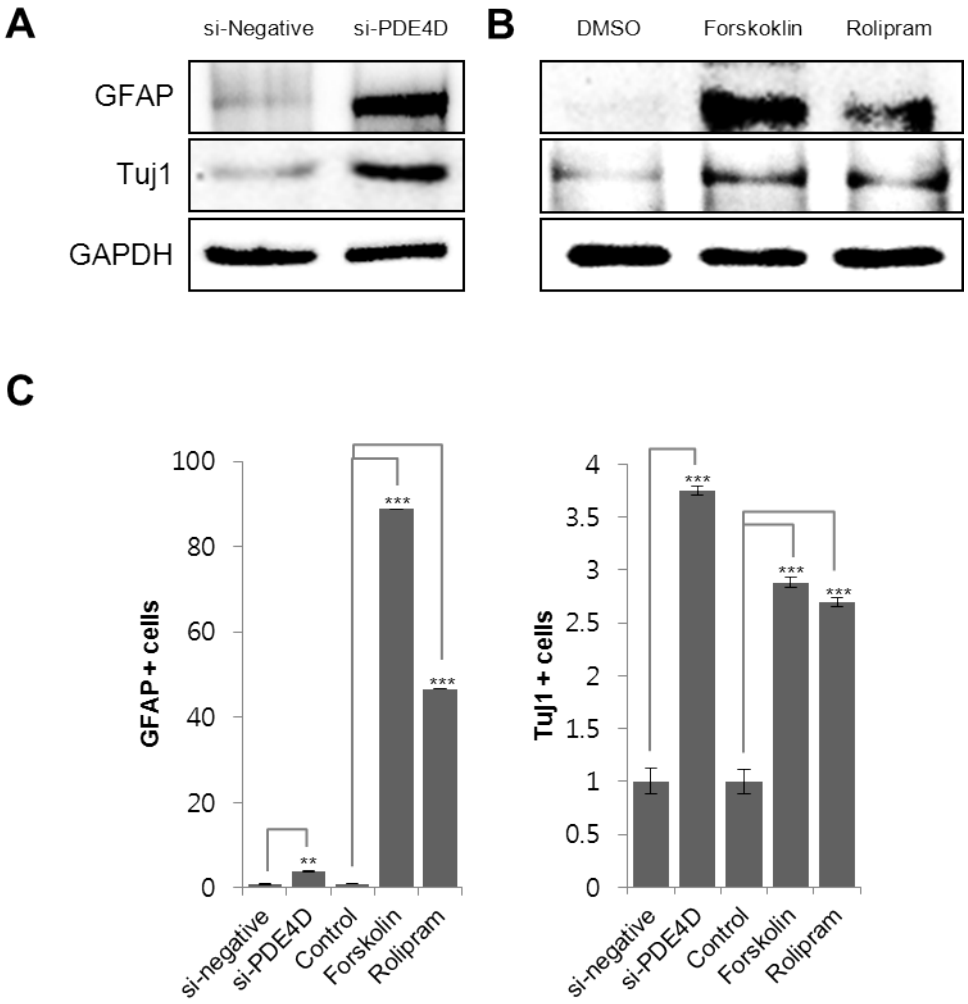

Figure 5. Si-PDE4D, Forskolin and Rolipram induce neural differentiation of GBM neurospheres

A,B) Western blot analysis show increased level of GFAP and Tuj1after treatment of si-negative, si-PDE4D, Forskolin and Rolipram. C) The expression of proteins were measured using Image J. Vehicle (=DMSO) was used for control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

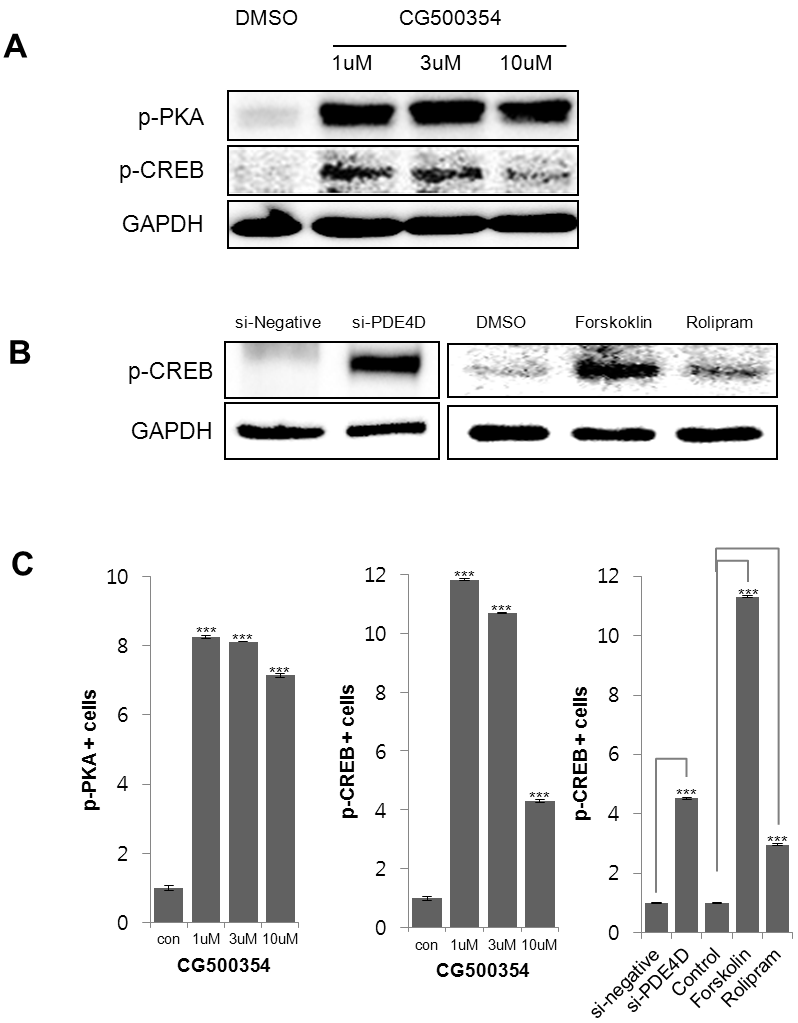

Figure 6. CG500354 and mimetic substances promote cAMP/PKA signaling pathway

A, B) Western blot analysis show increased level of p-PKA and p-CREB after treatment of CG500354 and si-negative, si-PDE4D, Forskolin as well as Rolipram. C) The expression of p-PKA and p-CREB proteins were measured using Image J. Vehicle (=DMSO) was used for control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

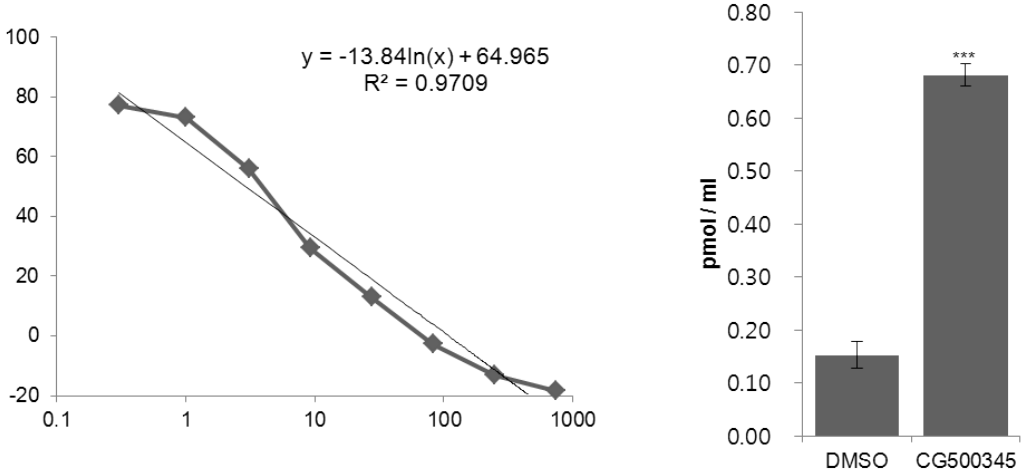

Figure 7. CG500354 actives cAMP secretion

The data showed cAMP secretion level from the supernatant after 72 hours of CG500354 treatment. The concentration of cAMP was measured by Cyclic AMP EIA Kit.

*, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

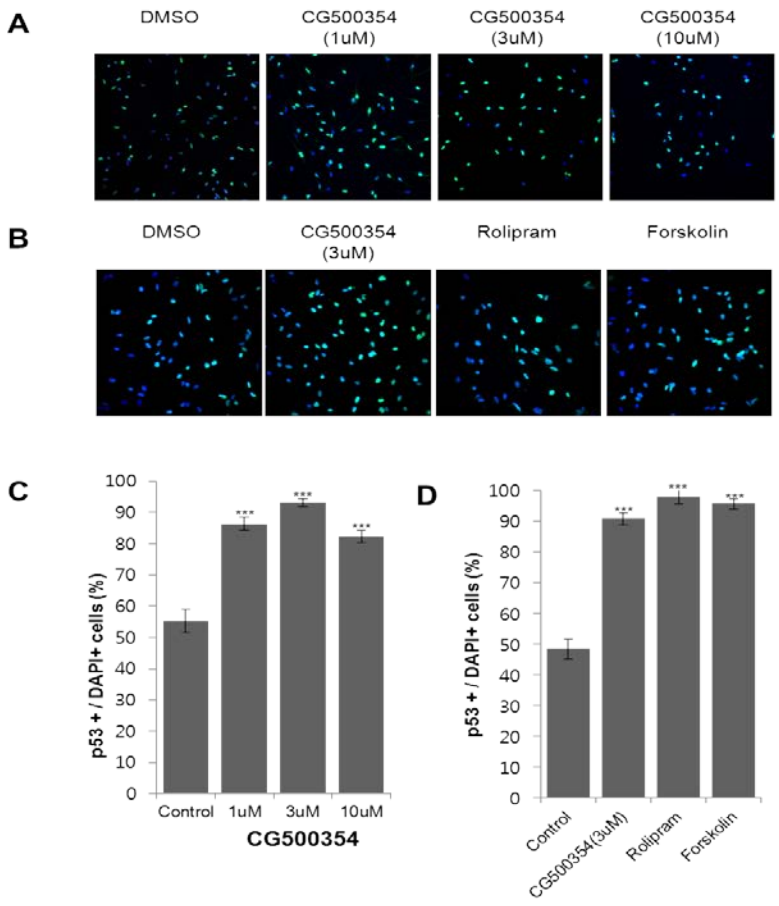

Figure 8. CG500354, si-PED4D, Forskolin and Rolipram increase the number of p53-expressing cells

A,B) Immunocytochemistry analysis of GBM cells treated with 1uM, 3uM and 10uM CG500354, Rolipram and Forskolin show increased expression of p53 marker. Nuclei counterstained in blue with DAPI. C,D) The efficiency of p53-expressing cells from A, B was calculated. These graphs indicate percent of p53+ cells. Vehicle (=DMSO) was used for control. *, $P < 0.05$; **, $P < 0.01$;***, $P < 0.001$.

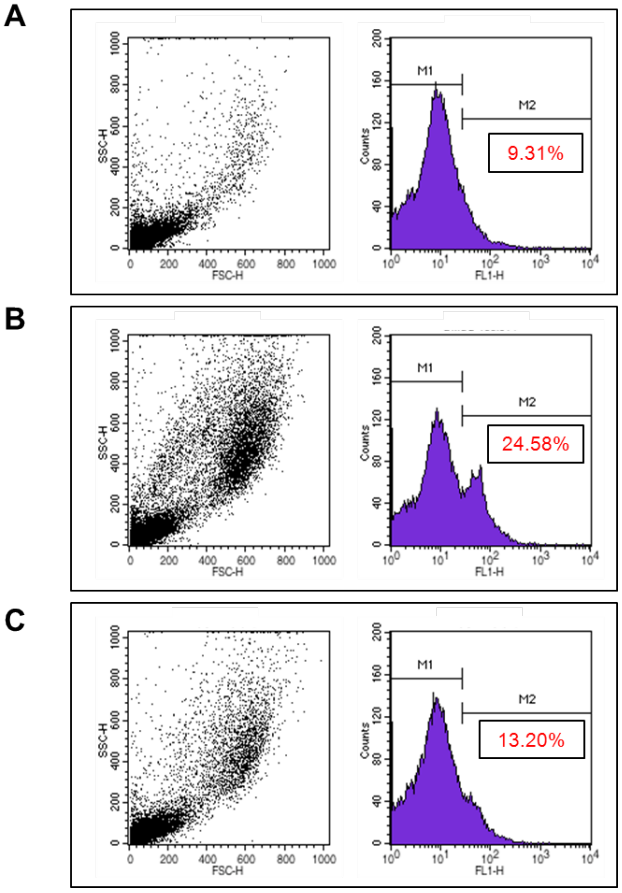

Figure 9. CG500354 targets CD133-expressing subpopulation

Flow cytometry analysis was performed to classify a subpopulation. A) Negative control B) DMSO treated GBM stained with CD133 antibody C) CG500354 treated GBM stained with CD133 antibody.

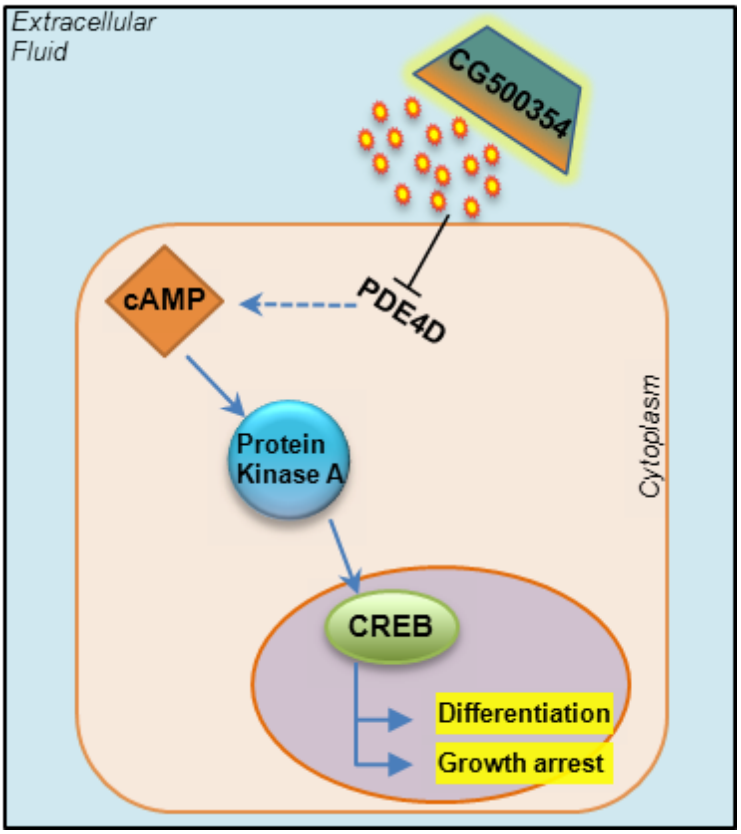

Figure 10. Scheme of cAMP/PKA signal cascade

Neural differentiation and growth arrest of GBM cells were induced through cAMP/PKA pathway by CG500354 treatment.

DISCUSSION

In this study, we determined the biological effect of CG500354, PDE4D inhibitor on human derived GBM CSLSs. Interestingly, CG500354 accelerated the neural differentiation of GBM cells which accompanied growth arrest and stemness attenuation. It has been well accepted that CSLCs in various tissues play a pivotal role in the development and progression of cancers. Regarding to role of CSLCs, recent evidences are emerging that CSLC differentiation will attempts to end of the cycle of self-renewal.

Indeed, PDE4D inhibitor CG500354 induced the differentiation of GBM, however the neural differentiation led cells to undergo itself to decrease the endogenous self-renewal capabilities. cAMP-dependent pathway is widely known as a cellular pathway involving in regulation of cell proliferation/growth arrest, differentiation and apoptosis (Lee, 1999). When cAMP signaling was accelerated, it augments the apoptosis in cancer cells. In a reliable report about cAMP roles, PDE4 appeared the most important target for induction of apoptosis and differentiation in leukemia cells (Copsel et al., 2011; Karin, 1994).

In agreement with the previous reports, our findings suggest that the permanent cAMP stimulation induces growth arrest via phosphorylation of PKA/CREB in GBM. Moreover, we have shown that the cAMP signaling pathway through PDE4D inhibition led GBM CSLCs to differentiate and lose their tumorigenic potential and stemness by down-regulating Nestin. Rolipram, PDE inhibitor just ended up with being memory enhancer. However, CG500354 targeting specific variant PDE4D showed further feature of closer step to therapeutic issues of aggressive glioblastoma. It is now clearly known yet the

variants of PDE4 and used for an anti-cancer effect out of more than 20 PDE variants, it could be the novel issue to us to inferred that if we maximize cell growth arrest and differentiation related regulator cAMP pathway potential could be a breakthrough to create a clinical therapy to this aggressive neoplastic glioblastoma cells. In this study, we have shown that the endogenous knockdown of PDE4D was similarly appeared the treatment with CG500354 in GBM and it was associated with up-regulation of cAMP/PKA signaling.

This is consistent with other paper which represent about cell growth arrest and differentiation via cAMP/PKA. Taken together, our findings suggest that CG500354, novel PDE4D inhibitor in cAMP/PKA signaling, induced neural differentiation as well as growth arrest via induction of PKA and CREB following CG500354-mediated cAMP up-regulation (Fig. 10). Therefore, the novel targeting for PDE4D might provide better understanding to develop new drugs for GBM in near future.

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국 문 초 록

초대다형성교아종에서의 cAMP/PKA 신호경로를 통한 세포주기억제와 신경분화를 유도시키는 인산디에스테라아제-4 아형 D 억제제에 관한 연구

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다형성교아종 (Glioblastoma Multiforme)은 많은 의학적 치료들에 저항성이 있어 성인에서 일년 이하의 생존율을 보이는 가장 잘 알려진 악성 뇌종양이다. 최근 연구 결과들은 교아종의 매우 작은 부분을 차지하는 CD133 양성을 띄는 암 줄기 세포가 교아종의 시발점임을 보고해오고 있다.

비록, 싸이클릭 AMP 인산디에스테라아제-4 (PDE4) 억제제가 강력한 항암제로 여겨지고는 있지만 인산디에스테라아제-4의 억제가 어떻게 교아종에서 항암적 영향을 조절하는지는 아직 잘 알려져 있지 않다. 그리하여 본 연구

에서는 교아종에서 인산디에스테라아제-4 아형 D (PDE4D)의 억제 물질인 CG500354를 조사하고 이 물질의 능력과 세포신호 경로를 밝혔다. CG500354는 p21과 p27의 mRNA 발현을 증가시키는 동시에 싸이클린 B1을 감소시킴에 따라 교아종의 성장을 현저히 감소 시켰다. 면역세포화학 염색에서 또한 성상세포 마커, GFAP와 신경계 마커 Tuj-1의 발현이 증가함과 동시에 신경간세포 마커인 Nestin이 현저히 감소하였다. 인산디에스테라아제-4 아형 D 가 cAMP/PKA 신호 경로에 관여함에 따라 인산화단백질키나아제 A (p-PKA)와 인산화cAMP반응순서결합단백질 (p-CREB)의 단백질 발현 정도를 확인해보았다. 특수 단백질 검출 검사를 통해 CG500354가 면역세포화학 염색 결과처럼 GFAP, Tuj-1의 단백질 발현이 증가하였으며 더 나아가 p-PKA와 p-CREB의 발현 또한 현저히 증가하였다. 위 결과는 cAMP 측정 분석을 통해 다시금 확인되었다. 위의 결과들은 cAMP 측정법을 통하여 확인 및 지지되었으며, 면역세포화학 염색에서도 CG500354가 p53 발현 세포를 증가시켰다. 종합적으로, CG500354는 세포주기, 종양억제 마커 그리고 신경 분화 마커들의 레벨을 증가시킴으로 GBM의 성장 억제를 조절하는 중요한 역할을 한다.

주용어 : 교아종, 인산디에스테라아제-4 아형 D 억제제, cAMP, 신경계분화, 항암

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