Malignant gliomas can be converted to benign glial cells through treatment with a combination of small molecules

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Directed by Professor Yoon Ha

The Master's Thesis
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This certifies that the Master's Thesis
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ABSTRACT

Malignant gliomas can be converted to benign glial cells through treatment with a combination of small molecules

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Gliomas, the most malignant central nervous system tumors, are known to have a very poor survival rate. Given that gliomas are derived from mutations of glial precursor cells, a considerable number of them still strongly react with glial-precursor-cell-specific markers. Thus, it was investigated whether malignant gliomas can be converted to glial cells through the regulation of endogenous gene expression implicated in glial precursor cells. In this study, three small molecule compounds (cyclic adenosine monophosphate (cAMP) enhancer, mechanistic target of rapamycin (mTOR) inhibitor and Bromodomain and Extra-Terminal motif (BET) inhibitor) were used to convert malignant glioma cells to benign glial cells. Small-molecule-induced gliomas (SMiGs) were not only transformed into the glial-specific morphology but also showed positive reactions with glial-specific markers such as GFAP, CNP, and RIP. A microarray analysis showed that SMiGs exhibited a marked increase of glial-enriched genes, whereas the malignant-cancer-cell-specific gene was

greatly decreased. Moreover, proliferation of malignant glioma cells could be greatly suppressed after being converted to benign glial cells.

These findings show the proof of concept that malignant gliomas can be reprogrammed to benign glial cells, using a combination treatment of small molecules, and their proliferation can be regulated by the differentiation. This study suggest that the small molecule composition (i.e., forskolin, rapamycin, and I-BET151) may be the next generation of anticancer agent, which acts by reprogramming malignant gliomas to differentiate into glial cells.

Key words: glioma, forskolin, rapamycin, I-BET151, glia differentiation

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Ⅰ. INTRODUCTION

Many studies have reported that mouse or human fibroblasts can be directly reprogrammed into neurons, neural stem cells, or glial precursor cells via introducing cell-specific transcription factors.[1-9] Malignant gliomas can also be converted into functional neurons by the introduction of neural-cell-specific transcription factors.[10,11] These studies provide the possibility that direct reprogramming technology can change a cell's fate, irrespective of the cell type.

Most direct reprogramming studies have used the lentivirus system to introduce cell-specific transcription factors into donor cells;[8] this may lead to insertion of the host chromosome. However, in a previous study, a direct reprogramming technology based on small molecule compounds was developed to overcome the critical problem of the virus platform.[12,13] This new technology made it possible to convert mouse and human somatic cells into neurons, neural stem cells, or glial cells without inserting the host chromosome. Therefore, these small molecule compounds can replace the role of the transcription factors in direct reprogramming.

Previous studies have reported that gliomas are derived from glial precursor cells,[14] and a significant portion of gliomas still strongly react with glial-precursor-cell-specific markers.[15] However, it has not been explicitly investigated whether gliomas can be transformed into benign glial cells. Therefore, it was hypothesized that regulating the glial-specific endogenous gene expression of gliomas, through treatment with a combination of small molecule compounds that regulate the signal pathway, would reprogram malignant gliomas into benign glial cells.

Ⅱ. MATERIAL AND METHODS

1. Cell culture

In this study, rat C6 glioma cells were used. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Hyclone, UT, USA), 0.1% β-mercaptoethanol (Life Technologies), 1% penicillin/streptomycin (P/S; Life Technologies), 1% non-essential amino acids (NEAA; Life Technologies), and 1% sodium pyruvate (Life Technologies) and incubated at 37℃ in a humidified atmosphere containing 5% CO_2.

2. Differentiation and maturation of glial cells

Glioma cells were seeded on 1% basement membrane matrix (BD Biosciences, San Jose, CA, USA)-coated plates at a density of 1×10^3 cells/cm^2. After incubating them at 37℃ for 24 hr, the glioma culture medium was replaced with glial differentiation medium, which consisted of neurobasal medium : advanced DMEM/F12 (1X) (1:1), 1% N2 supplement (100X), 2% B27 supplement (50X), 0.05% bovine serum albumin (BSA), 1% P/S, 1% glutamax-I (100X), 0.1% 2-mercaptoethanol (1000X) (all of which were from Life Technologies), BDNF (10 ng/ml), GDNF (10 ng/ml), and NT-3 (10 ng/ml) (all of which were from PeproTech, Rocky Hill, NJ, USA). Then, a combination of small molecules composed of either forskolin (Tocris Bioscience, Bristol, UK) and rapamycin (Sigma-Aldrich, St. Louis, MO, USA) or forskolin, rapamycin, and T3 (Sigma-Aldrich) was added. The small molecules were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and diluted in glial differentiation medium to the following final concentrations: forskolin, 100 μM; rapamycin, 100 nM; T3, 100 nM. The glioma cells were

incubated in the respective media at 37℃ for 7 days, with the media being refreshed every 2 days.

After 7 days, the existing combination of small molecules was replaced with another combination consisting of T3, rapamycin and I-BET151 (Tocris Bioscience) or only I-BET151 to mature the differentiated cells. I-BET151 was also dissolved in DMSO and diluted to a final concentration of 1 μM. The cells were then incubated in the new media at 37℃ for 7 more days, with the media being refreshed every 2 days.

3. MTT assay

For measuring cell proliferation, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) assay was performed. Cells from each group, each containing the same volume of DMSO as that in the differentiation media, were differentiated for 7 or 14 days in 24-well plates. Then, 5 mg/ml MTT solution was added to the media and they were incubated at 37℃ for 3 hr. The media were removed, and formazan crystals were dissolved using DMSO. The samples were then incubated at 37℃ for 10 min and transferred to 96-well plates, and the absorbance was measured at 490 nm using VersaMax ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA).

In some experiments, the temozolomide (TMZ, 50 μM) was used to compare the cell growth inhibition. At experiment day, MTT assay was performed as described above.

4. Immunofluorescence and cell quantification

Media were removed and the cells were washed with phosphate-buffered saline (PBS; Hyclone). Then, the cells were fixed with 4% paraformaldehyde

(Millipore Temecula, CA, USA), pH 7.2, for 10 min at room temperature. Subsequently, they were washed thrice with 0.3% Tween 20 (Life Technologies) in PBS for 3 min. The blocking procedure was performed for 30 min at room temperature. Primary antibodies diluted in the blocking buffer, namely, rabbit polyclonal anti-Nestin (Millipore; 1:1000), rabbit polyclonal anti-NG2 (Abcam, Cambridge, MA, USA; 1:250), rabbit polyclonal anti-Olig2 (Abcam; 1:100), rabbit polyclonal anti-PDGFRa (Santa Cruz; 1:500), mouse monoclonal anti-MBP (Abcam; 1:1000), mouse monoclonal anti-CNP (Abcam; 1:1000), mouse monoclonal anti-RIP (Millipore; 1:50000), rabbit polyclonal anti-GFAP (Abcam; 1:1000), mouse monoclonal anti-GFAP (Sigma-Aldrich; 1:1000), mouse monoclonal anti-O4 (Millipore; 1:100), and rabbit polyclonal anti-Ki67 (Abcam; 1:250), were allowed to react with the samples for 1 hr at room temperature. After wash, secondary antibodies diluted in the blocking buffer were added and allowed to react for 30 min at room temperature: FITC donkey anti-rabbit or anti-mouse and Cy3 donkey anti-rabbit or anti-mouse (all of them are from Jackson ImmunoResearch Laboratories, West Grove, PA, USA; 1:500) were used as secondary antibodies. Then, the samples were washed thrice and stained using 4′,6′-diamino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA, USA). The samples were then covered with glass coverslips and examined using a confocal laser scanning microscope (LSM 700; Zeiss, Oberkochen, Germany). The number of Ki67-positive and Ki67-negative cells, using Image J, was measured in three fields chosen randomly and these results were converted into a percentage.

5. Flow cytometry

To measure the positive cells stained by each antibody, flow cytometry analyses were performed. Briefly, cells were harvested using 0.25% trypsin/EDTA (Life Technologies). For fixation, 4% paraformaldehyde was added to the cell pellet and incubated for 10 min. After centrifugation at 2000 rpm for 5 min, the supernatant was discarded and the cell pellet was washed twice with 0.3% Tween 20 in PBS for 3 min. Then, the same primary antibodies used for immunofluorescence were added to the pellet and allowed to react for 1 hr at room temperature. Following a second wash using the same procedure, secondary antibodies were added to the cell pellet and allowed to react for 30 min at room temperature. Finally, the pellet was washed and resuspended in 200 μl 0.3% Tween 20 in PBS. Samples were analyzed using a FACSCalibur (Becton Dickinson, Rutherford, NJ, USA) and Cell Quest software (Becton Dickinson).

6. Microarray

Microarray analysis was performed according to the manufacturer's instructions. After total RNA isolation, cDNA was synthesized using the GeneChip WT (Whole Transcript) Amplification kit. Labeled DNA target was hybridized to the AffymetrixGeneChip Array. Hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner. Analysis was performed using Affymetrix® GeneChip Command Console® Software (AGCC). Microarray data have been deposited in a public database (https://www.ncbi.nlm.nih.gov/geo/), GSE101337 (For undifferentiated C6 and SMiG) and 101338 (For single clone #4 and single clone #8).

7. Cell counting

To determine the proliferation rate of untreated glioma cells, i.e., those without the small molecules, they were seeded at a density of 1×10^3 cells/cm^2 on a 24-well plate. When the cells reached confluence, they were harvested using 0.25% trypsin/EDTA. After being centrifuged at 1200 rpm for 3 min and re-suspended in 1 ml culture medium, the cells were diluted to half concentration in trypan blue (Life Technologies) and counted using the manual cell counting method. All of the cells were then seeded on 12-well plates (Coring). Again, when the cells reached confluence, they were counted and replated on wider plates, using the same method.

8. Single colony selection

For the single colony selection, 100 cells were mixed with 200 μl culture medium and placed into one well of a 96-well plate. Then, 100 μl was taken from the first well, transferred to the next well, and diluted to half using culture medium. This process was repeated multiple times, and the cells were cultured for 24 hr. When one cell was sufficiently isolated from the others, it was incubated until a colony was formed. This colony was removed from the 96-well plate, and then seeded on a 60 mm dish.

9. Statistical analysis

Student's t-test was performed to assess differences between two groups. Two-way analysis of variance (ANOVA) was performed to assess differences among three groups or more. Data are presented as the mean ± standard error of the mean (SEM) and *p*-values less than 0.05 were considered to be significant statistically.

Ⅲ. RESULTS

1. Direct conversion of malignant glioma cells into benign glial cells and their growth inhibition by treatment with a combination of small molecules

Here, it was confirmed that undifferentiated glioma cells were positive for specific makers of glial progenitor cells, namely, Nestin, NG2, Olig2, and PDGFRa (Fig. 1A). However, the glioma cells were negative for other makers of glial cells, namely, GFAP, CNP, RIP, and O4 (Fig. 1A).

Therefore, it was first examined whether T3, which is typically used to differentiate glial progenitor cells into oligodendrocytes, can convert gliomas into glial cells (i.e., oligodendrocytes). However, the glioma cells treated with T3 were not transformed into glial-specific morphology (Fig. 1B). Next, T3 and forskolin were added to the glioma cells, but glial-specific morphology wasn't confirmed after 7 days of incubation (Fig. 1B). Nevertheless, trying to convert C6 glioma cells into glial cells by adding rapamycin to the T3 and forskolin and incubating the cells for 7 days was continued. Finally, glial-specific morphology as well as glial-specific markers such as GFAP, CNP, and RIP was observed (Fig. 1B, 1C).

After various experiments were performed, it was then confirmed that this glial-specific morphology could be maintained using medium containing rapamycin, T3, and I-BET151 (Fig. 1D, E). To further confirm whether the converted cells were fully differentiated into glial cells, they were cultured in the absence of small molecules for 3 or more days. Consequently, it was observed that the glial-specific morphology was retained, and the GFAP-, CNP-, and RIP-positive cells did not react with Ki67 (Fig. 1F). Next, the effect of growth inhibition was investigated. The undifferentiated glioma cells grew

rapidly despite a very low cell density (Fig. 1G). In contrast, the treatment with a combination of forskolin, T3, and rapamycin conspicuously decreased the cell proliferation (Fig. 1H). Further experimentation confirmed that I-BET151 plays a major role in growth inhibition after glial induction (Fig. 1I).

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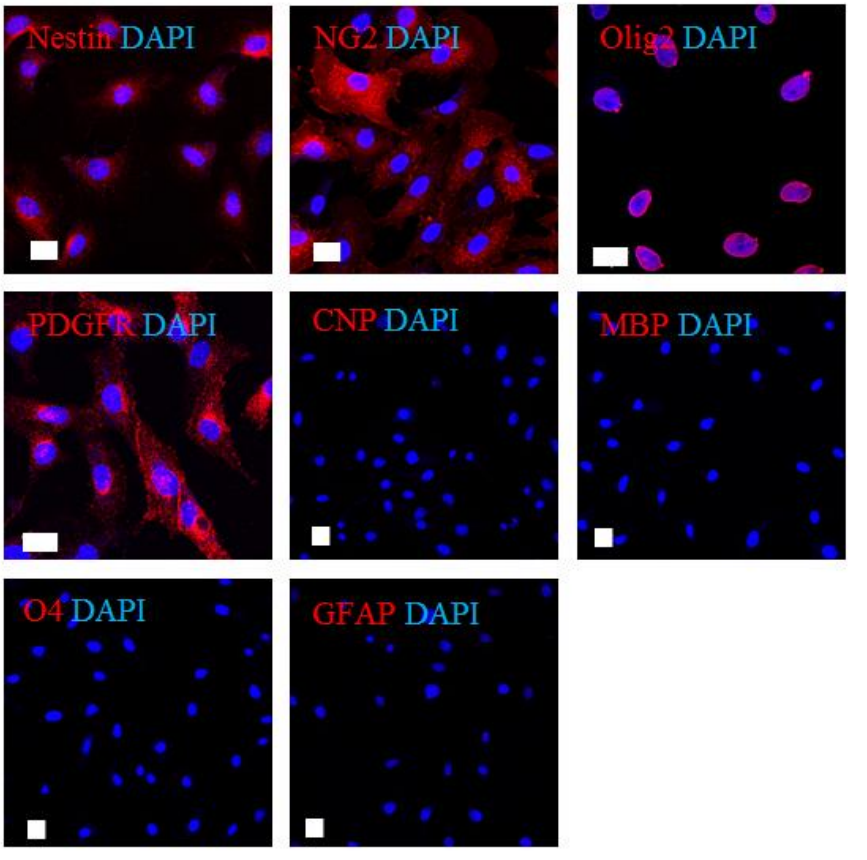

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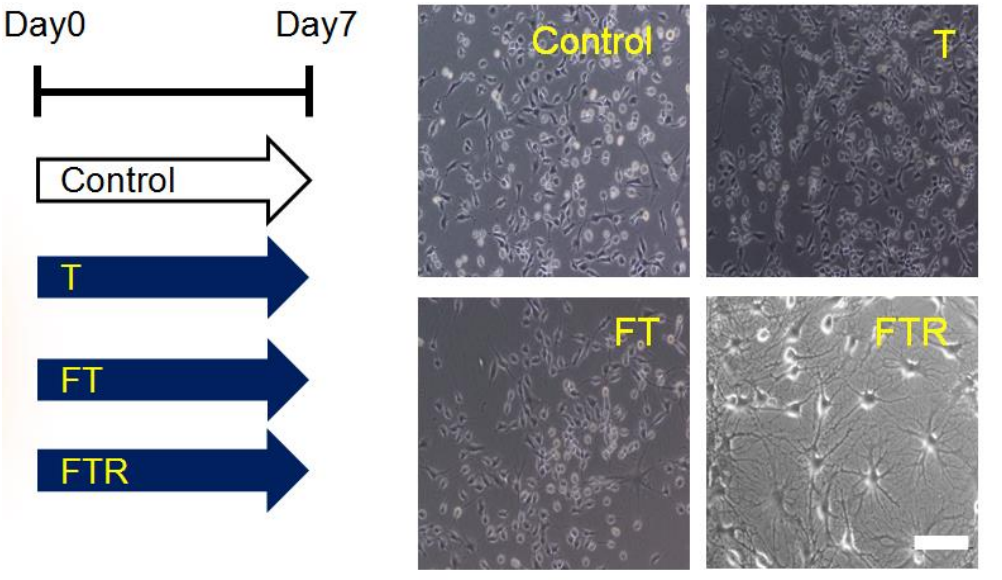

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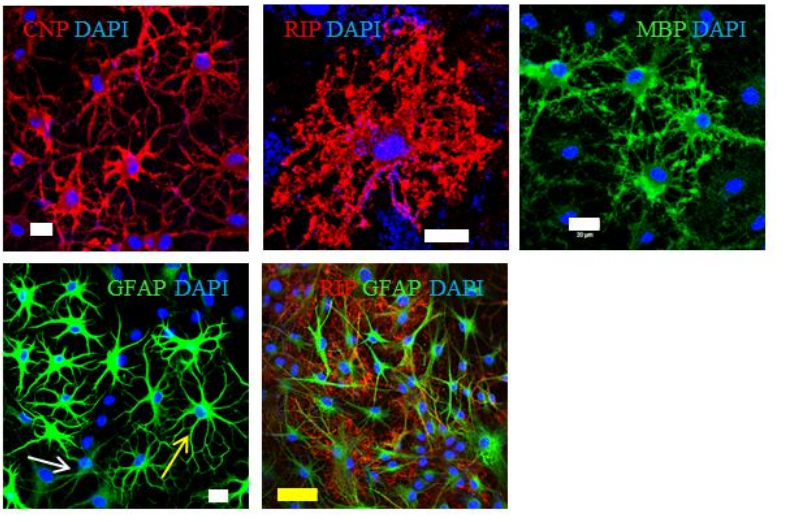

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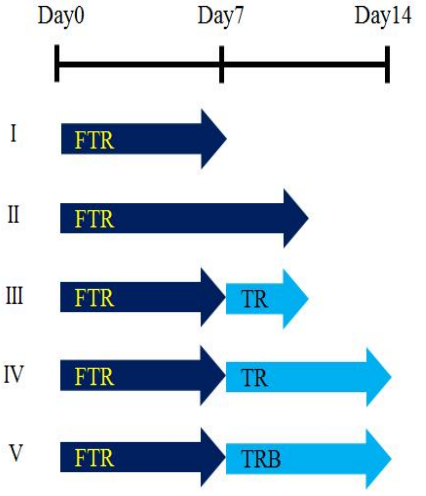

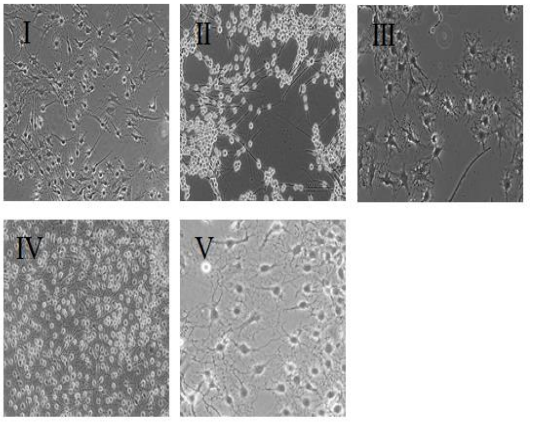

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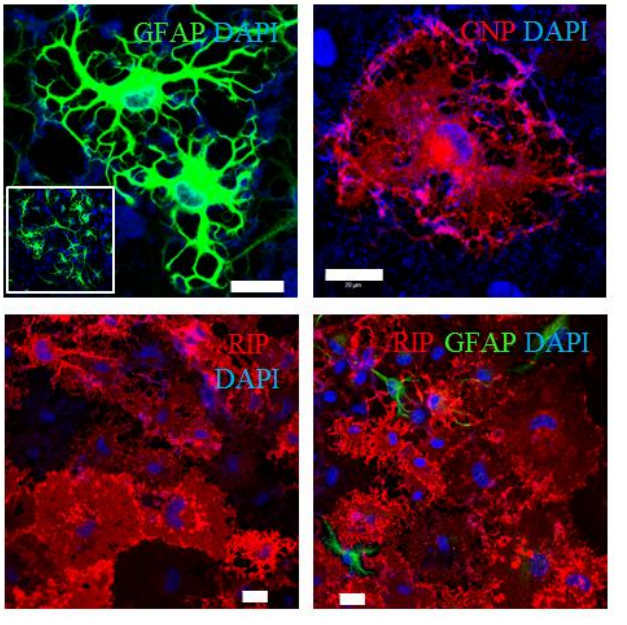

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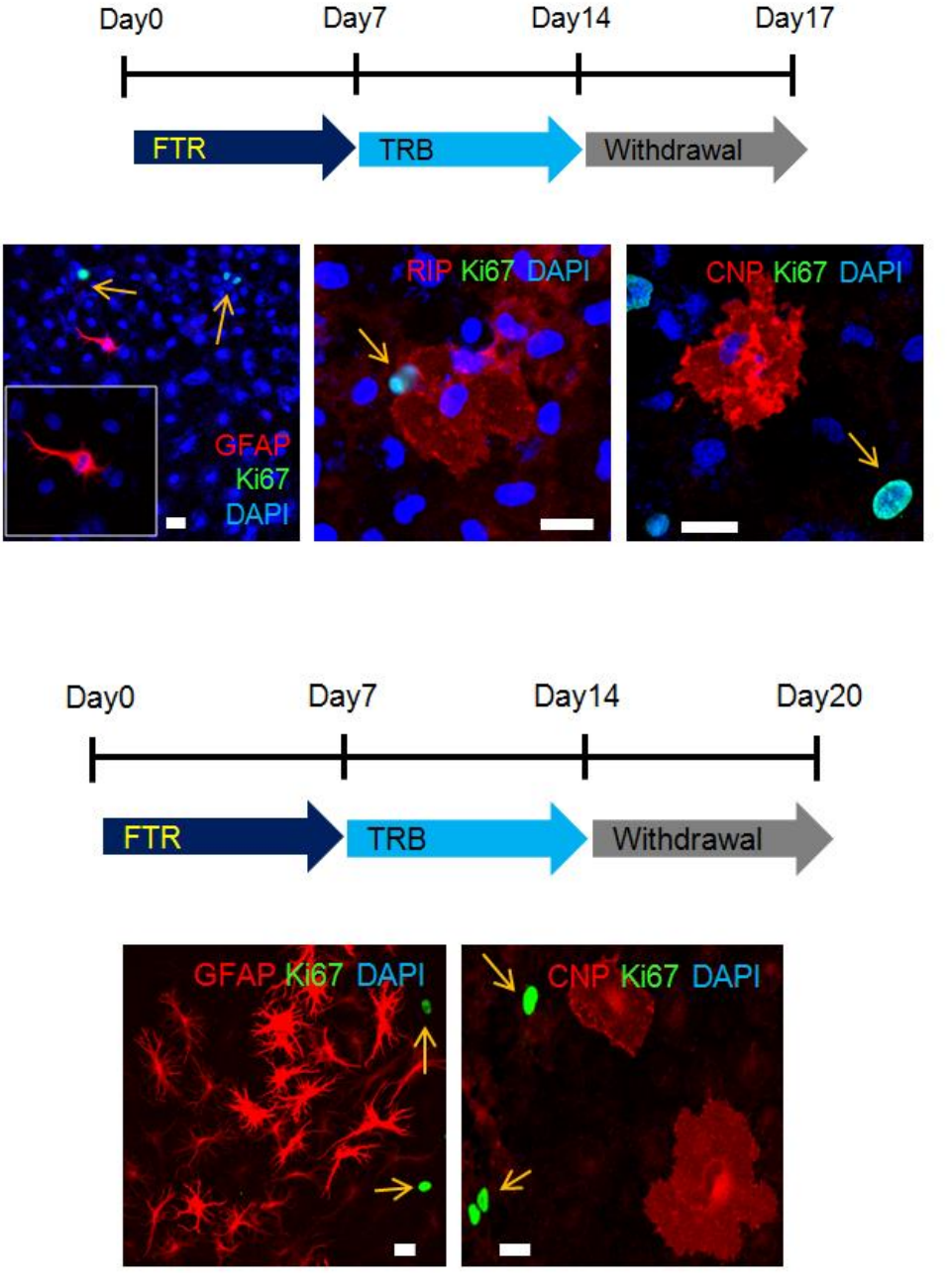

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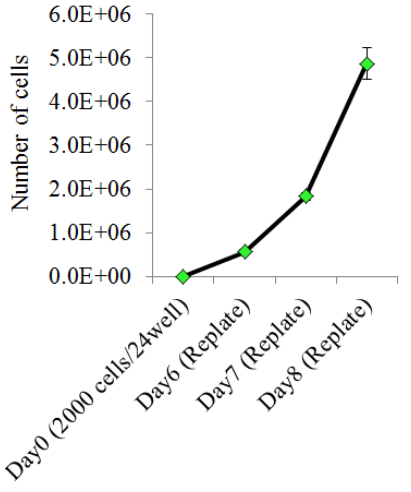

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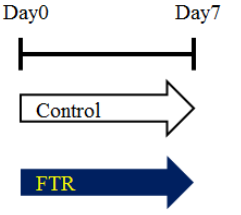

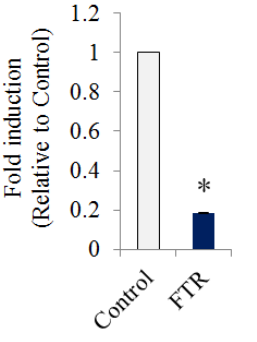

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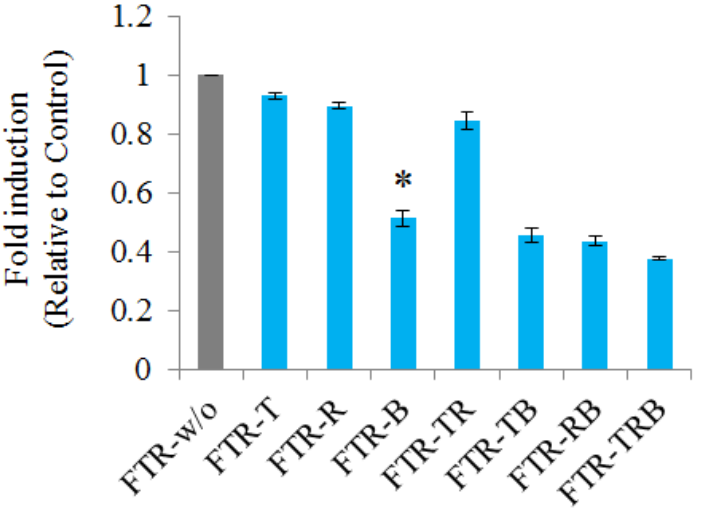

Figure 1. Glial differentiation of malignant glioma cells through treatment with small molecules. (A) Representative image stained with Nestin, NG2, PDGFR, Olig2, GFAP, CNP, RIP, and O4 in undifferentiated glioma cells. Scale bars represent 20 μm. (B) Representative image 7 days after induction of glial differentiation. T (T3), FT (forskolin/T3), FTR (forskolin/T3/rapamycin). Magnification (100x), Scale bars represent 100 μm. (C) Representative image stained with GFAP, CNP, and RIP 7 days after induction of glial differentiation. Scale bars represent 20 μm (White) and 50μm (Yellow). (D) Representative image after induction of glial differentiation with each different procedure. Magnification (100x). (E) Representative image stained with GFAP, CNP, and RIP 14 days after induction of glial differentiation with procedure V. Scale bars represent 20 μm. (F) Representative image stained with Ki67, GFAP, CNP, and RIP with 3 days and 5 days of small molecule withdrawal after glial differentiation for 14 days. Scale bars represent 20 μm. (G) Quantitative result of cell proliferation in differentiation media. (H) Quantitative result of cell density 7 days after induction of glial differentiation. (I) Quantitative result of cell density 14 days after induction of glial differentiation within each different combination of small molecules.

* indicates $p < 0.05$. Data are presented as the mean ± S.E.M.

2. Glial differentiation of malignant glioma cells through optimal treatment with small molecules

Next, further experiments were carried out to determine the major compound for glial induction among forskolin, T3, rapamycin and I-BET151. It was confirmed that glioma cells were converted into glial-specific morphology, and GFAP-, CNP-, RIP-, and O4-positive cells even in the absence of T3 (Fig. 2A, B). These converted cells did not react with Ki67 (marker for dividing cells) (Fig. 2B). Thus, T3 did not affect the glial conversion of the glioma cells.

It was further investigated whether glial-specific morphology can be maintained after induction of glial differentiation for 7 days. It was difficult to maintain glial-specific morphology, only using forskolin and rapamycin (Fig. 2C). In contrast, glial-specific morphology could be maintained in the presence of I-BET151 after enough induction of glial differentiation, using forskolin and rapamycin (Fig. 2D).

As a quantitative result, the number of CNP-, RIP-, and GFAP-positive cells clearly increased in SMiGs converted using forskolin, rapamycin, and I-BET151 (Fig. 2E). Also, it was observed that glial-specific morphology was maintained when small molecules were withdrawn for 3 days (Fig. 2F).

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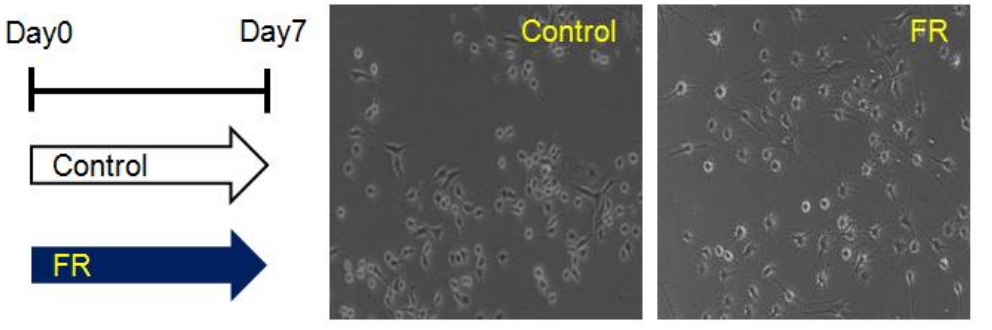

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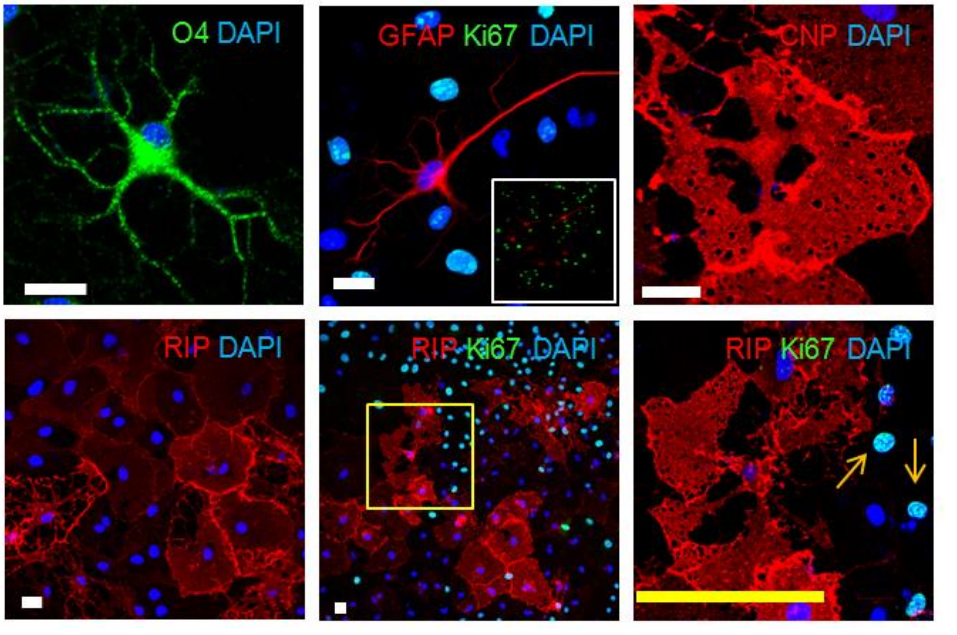

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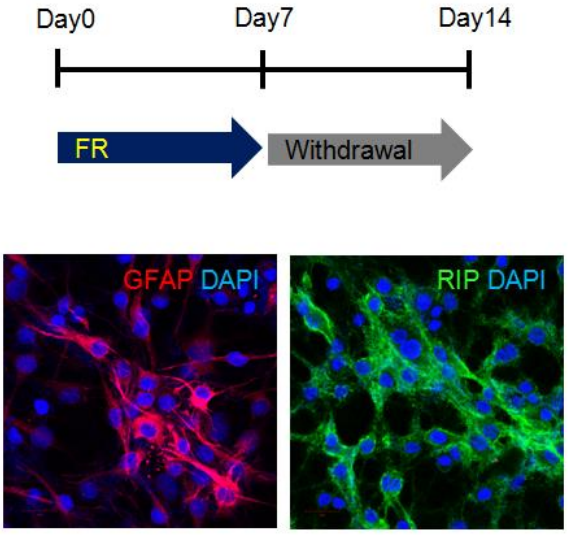

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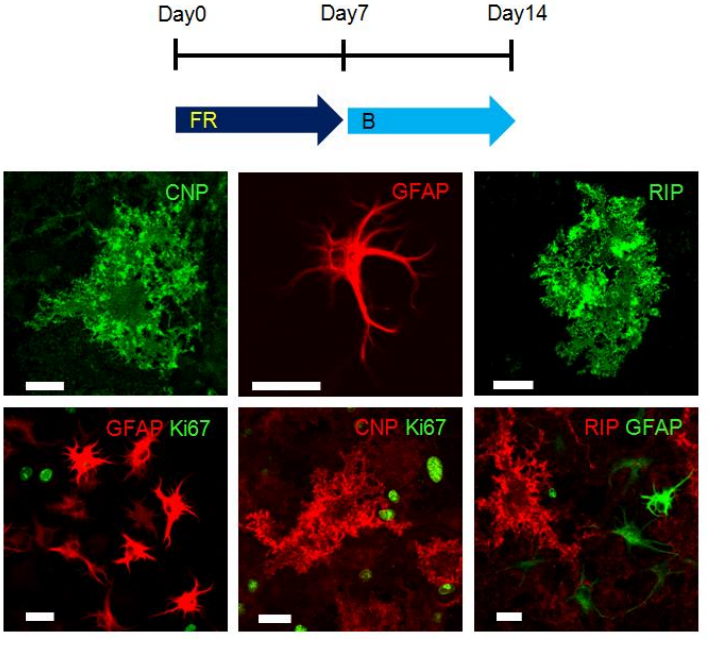

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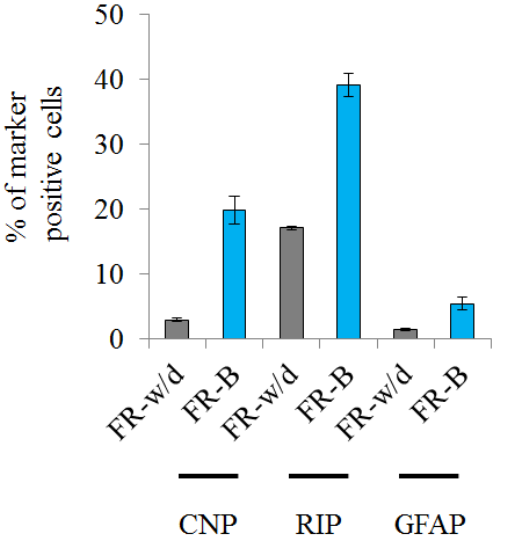

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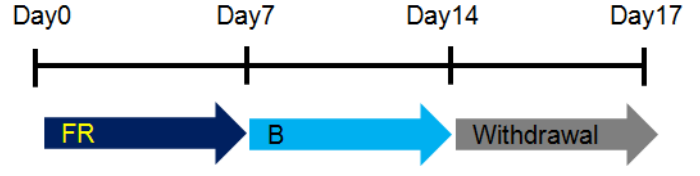


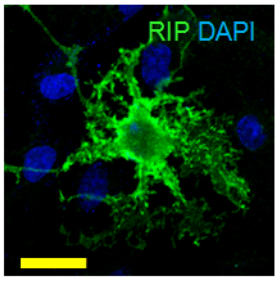
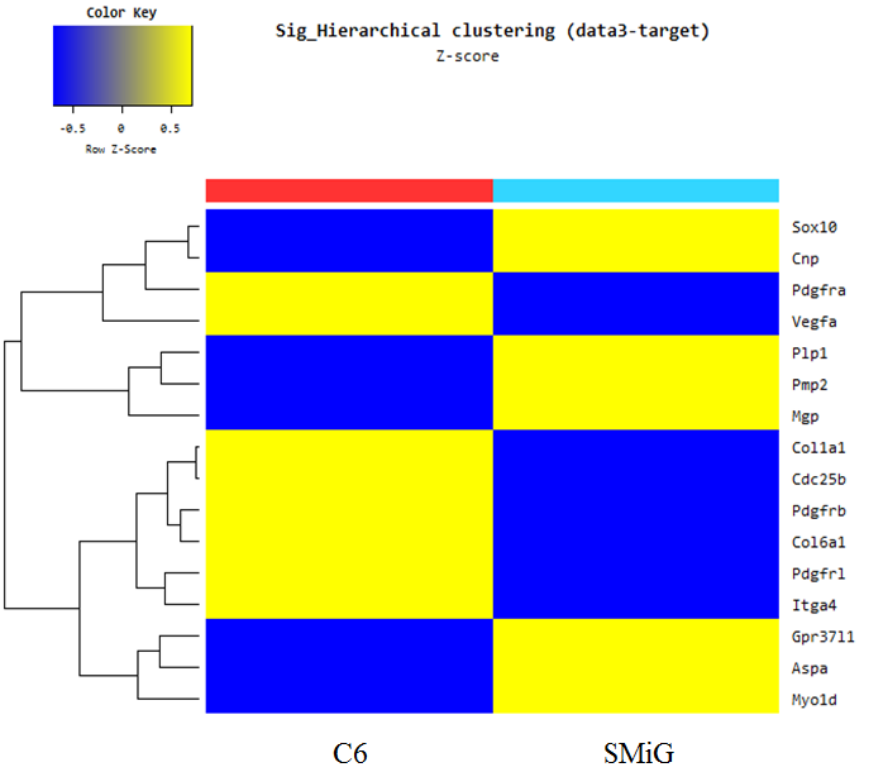


Figure 2. Glial differentiation of malignant glioma cells through optimal treatment with small molecules. (A) Representative image 7 days after induction of glial differentiation. Magnification (100x). (B) Representative image stained with GFAP, CNP, RIP, O4, and Ki67 7 days after induction of glial differentiation. Scale bars represent 20 μm (White) and 100μm (Yellow). (C) Representative image stained with GFAP, CNP, RIP, and Ki67 14 days after induction of glial differentiation. Scale bars represent 20 μm. (D) Representative image stained with GFAP and RIP with 7 days of small molecule withdrawal after induction of glial differentiation for 7 days. Scale bars represent 50 μm. (E) Quantitative result of the positive cells with the glial-specific marker after glial differentiation for 14 days by using flow cytometry. (F) Representative image stained with RIP with 3 days of small molecule withdrawal after glial differentiation for 14 days. Scale bars represent 20 μm (Yellow).

3. Gene expression profile in undifferentiated glioma cells and SMiGs

Lastly, microarray analysis was performed to compare the gene expression pattern between glioma cells and SMiGs. In a microarray analysis, gene expression profiling related to oligodendrocyte differentiation and myelination was significantly induced in SMiGs (Fig. 3A). In contrast, gene expression profiling relating to cell division and mitosis, extracellular matrix (ECM) and vessel development were markedly up-regulated in glioma cells (Fig. 3A). Gene ontology related to glial differentiation, including oligodendrocyte differentiation and myelination, was significantly different between undifferentiated glioma cells and SMiGs (Fig. 3B). These results mean that the specific characteristics of malignant gliomas can be converted into those of benign glial cells by treatment with a combination of forskolin, rapamycin, and I-BET151.

A



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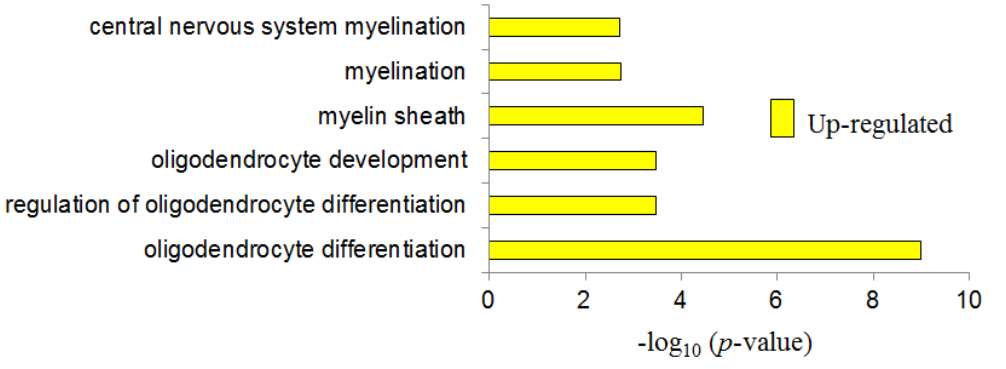
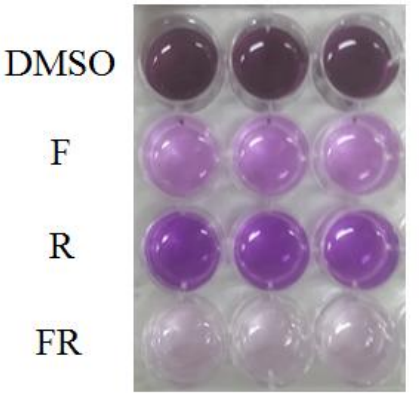


Figure 3. Comparison of gene expression pattern between glioma cells and SMiGs. (A) Up- or down-regulated gene lists in glioma cells and SMiGs. (B) Up-regulated gene ontology term relating glial differentiation in SMiGs.

4. Proliferation of malignant glioma cells can be stopped by a combination of small molecules

It was investigated whether the strong proliferation potency of malignant gliomas can be inhibited by glial conversion. On day 7 after treatment with a combination of forskolin and rapamycin, it was confirmed that the proliferation of glioma cells was significantly reduced compared to that of the single-molecule treatment group (Fig. 4A, B), and the rate of Ki67-positive cells significantly decreased compared to that in the DMSO group (Fig. 4C). Further treatment with I-BET151, after glial induction with forskolin and rapamycin, was more effective to inhibit cell proliferation (Fig. 4D). Moreover, this pattern was similar even in the absence of the small molecules (Fig. 4E). These results indicate that the strong proliferation capacity of malignant glioma cell can be controlled by glial conversion.

A



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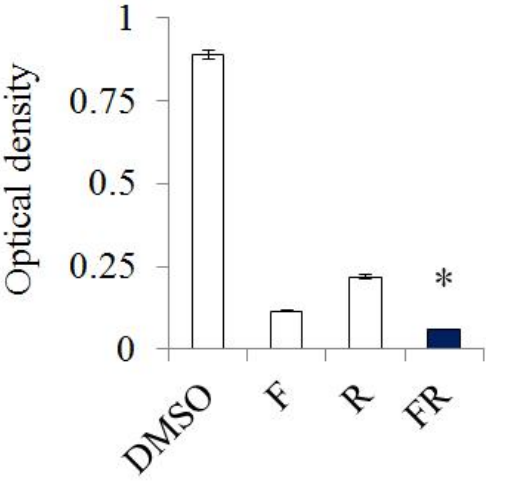

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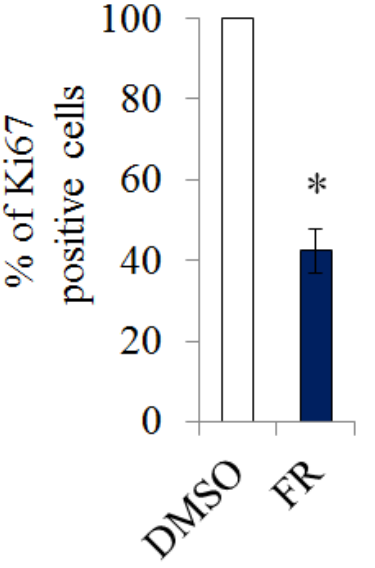

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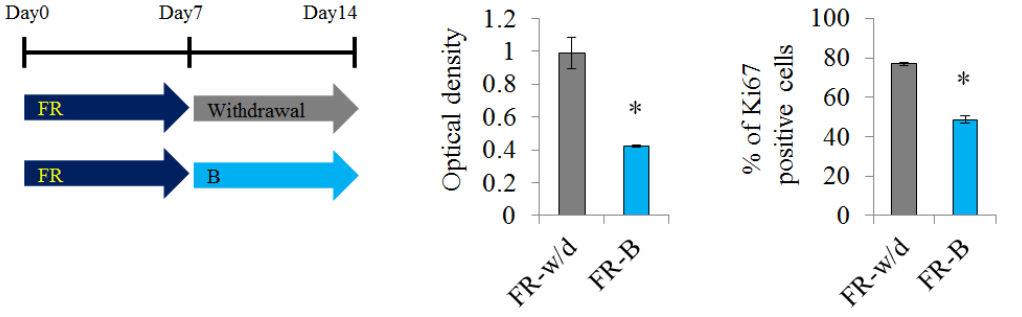

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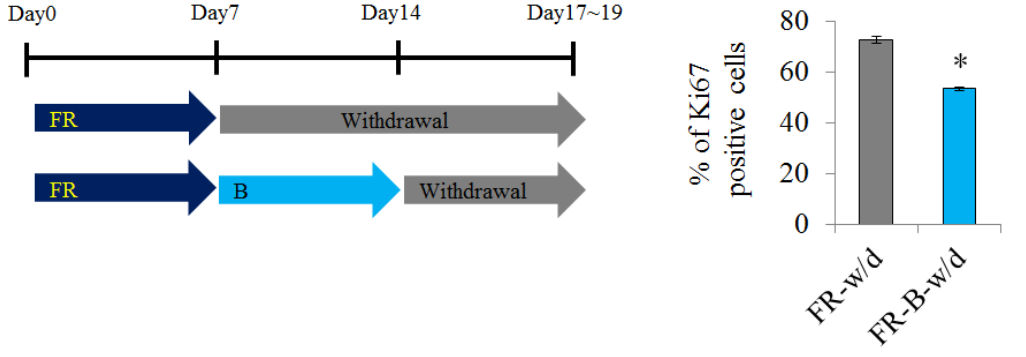


Figure 4. Effect of proliferation-inhibition of glioma cells by a combination of small molecules. (A) Representative image of formazan crystals dissolved using DMSO 7 days after induction of glial differentiation. (B) Quantitative results of cell density at day 7 after induction of glial differentiation obtained by using MTT assay. (C) Quantitative result of Ki67-positive cells 7 days after induction of glial differentiation through flow cytometry. (D) Quantitative results of cell density 14 days after glial differentiation and Percentage of Ki67-positive cells 14 days after glial differentiation. (E) Percentages of Ki67-positive cells 3 days after withdrawal of the small molecule compounds after glial differentiation for 14 days.

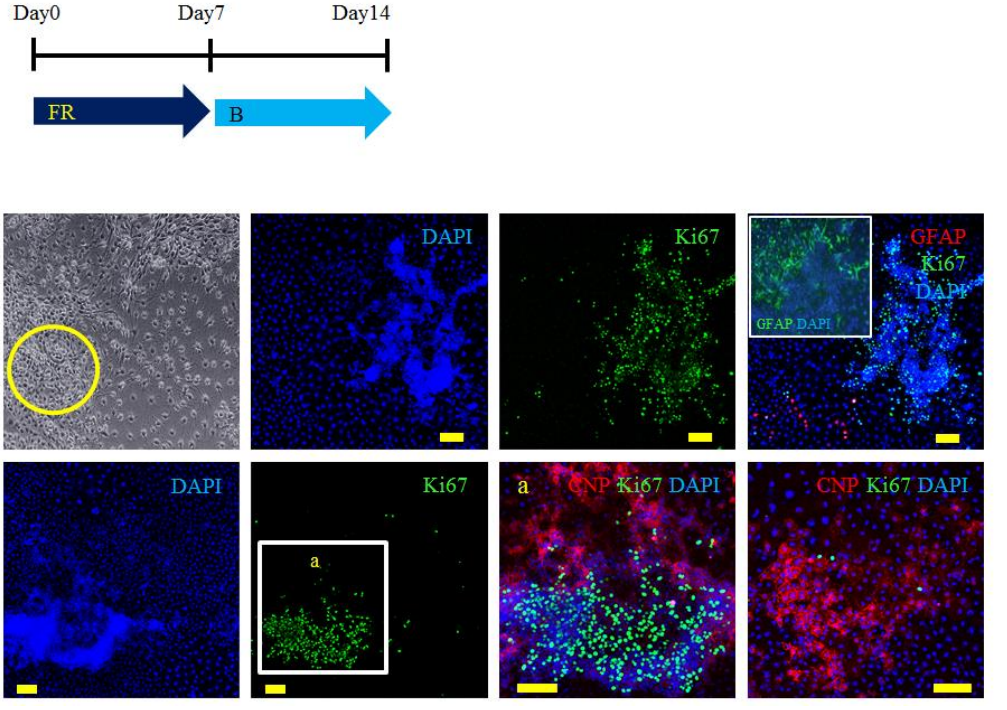
* indicates $p < 0.05$. Data are presented as the mean ± S.E.M.

5. Specific-cell type responding to the small-molecule combination for glial conversion

Approximately 40% of cells remained positive for Ki67 even in SMIGs (Fig. 4C). The colonies of these cells were not positive for GFAP, CNP (Fig. 5A). Therefore, each single colony with regards to the glioma's heterogeneity was selected (Fig. 5B). Of the many colonies isolated, colony No. 4 did not react with GFAP, CNP, or RIP after glial induction with forskolin, rapamycin and I-BET151; it was only positive for Ki67 (Fig. 5C).

However, the many of colony No. 8 were positive for GFAP, CNP, and RIP after glial induction with forskolin, rapamycin and I-BET151 (Fig. 5C). Also, GFAP-, CNP- and RIP-positive cells did not react with Ki-67 (Fig. 5C). And then, the gene expression patterns between colony No. 4 and 8 were analyzed, using a microarray analysis (Fig. 5D). The top 10 most-expressed genes in colony No. 8 were *prkcb, postn, mpz, pros1, cd55, ct55, plxdc2, antxr1, fmr1nb, igfbp5* (Fig. 5E, F). Moreover, the proliferation-inhibition effects of the small-molecule combination and a standard medication such as temozolomide (TMZ) were compared. For colony No. 4, cell proliferation was reduced by TMZ, but not by the small-molecule combination (Fig. 5G). In contrast, the small molecule combination inhibited proliferation more than TMZ in colony No. 8 (Fig. 5G).

A



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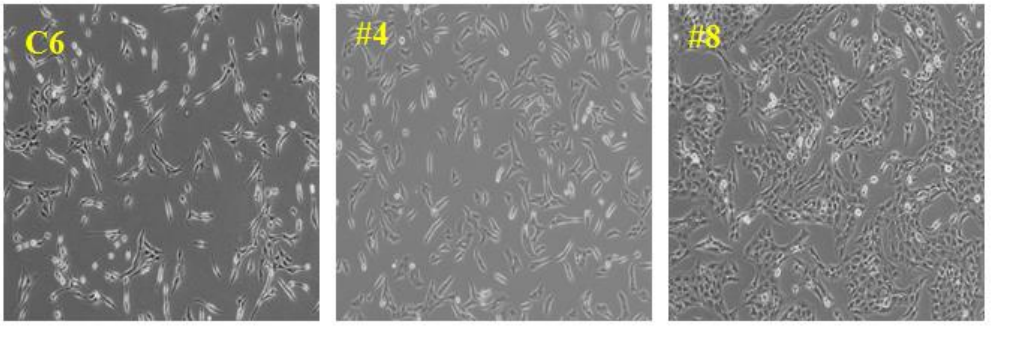

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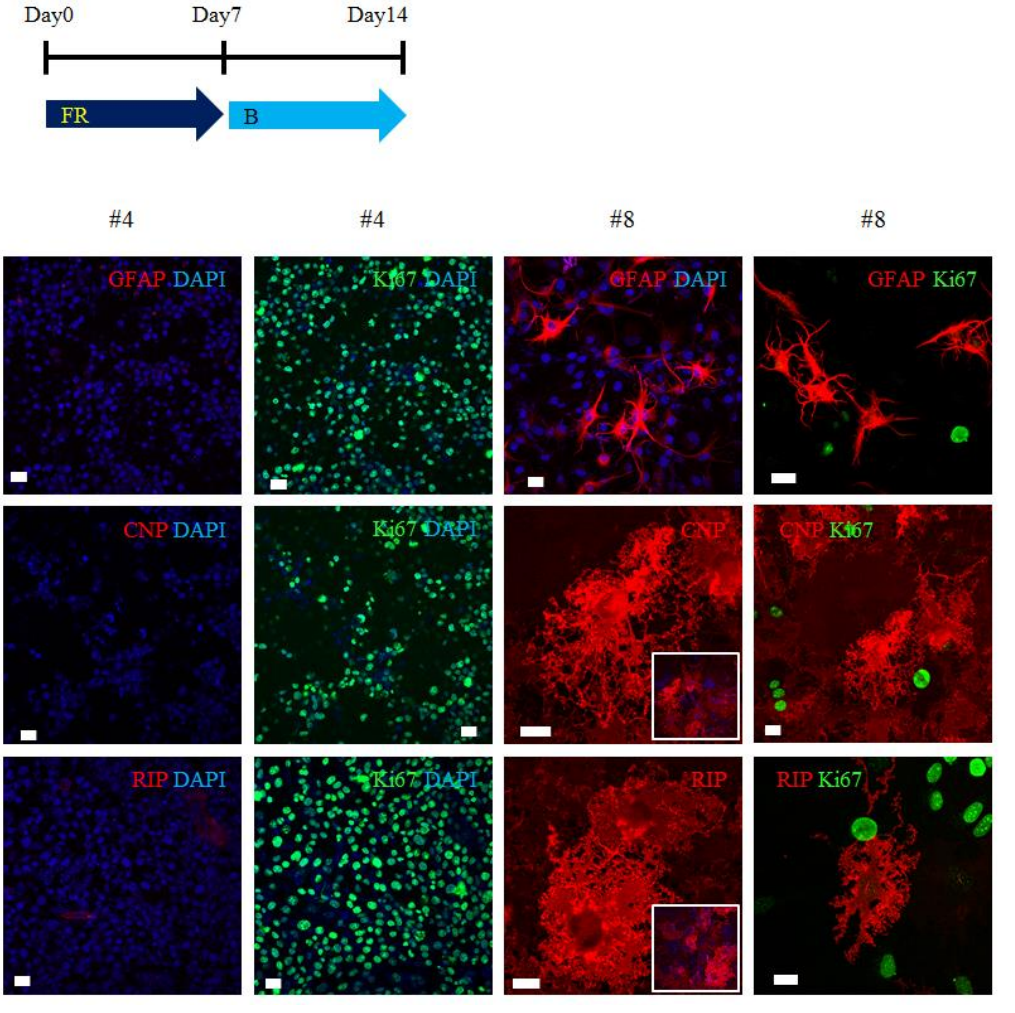

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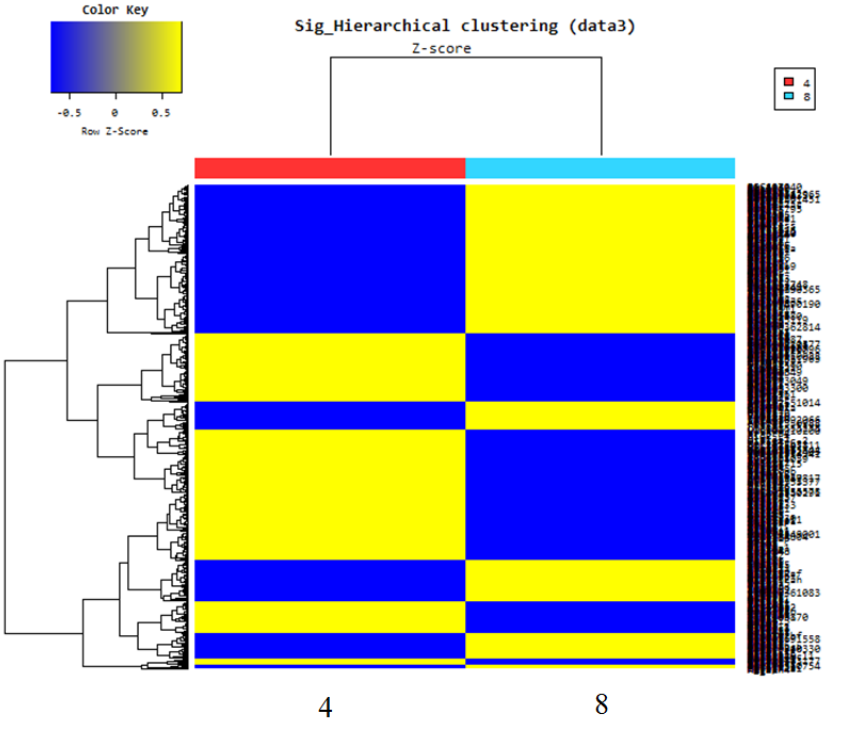

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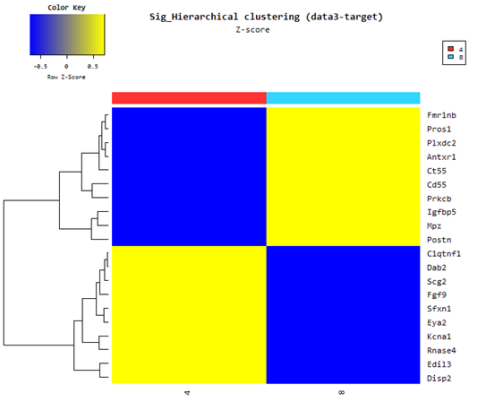

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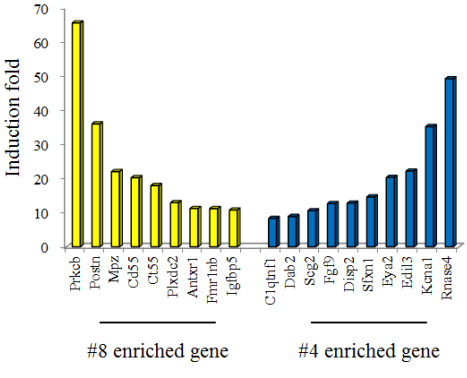

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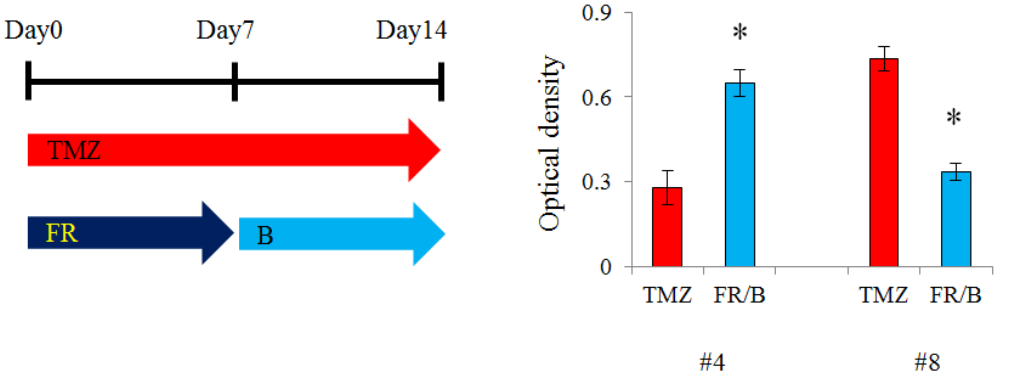

Figure 5. Effect of the glial-differentiation–based drug in each single clone derived from the glioma. (A) Representative image stained with Ki67/GFAP or Ki67/CNP in glioma cells. Scale bars represent 200 μm. (B) Representative image of single clones derived from glioma cells. (C) Representative image stained with Ki67/GFAP, Ki67/CNP, and Ki67/RIP 14 days after glial differentiation using forskolin, rapamycin, and I-BET151. Scale bars represent 20 μm. (D) Gene expression profile of two different single clones derived from glioma cells. Heatmap of the significant gene in single clones derived from glioma cells. (E) Top 10 gene expressions in single clones derived from glioma cells. (F) Induction fold of top 10 gene expression in single clones derived from glioma cells. (G) Quantitative results of cell density 14 days after treatment with TMZ or the glial-differentiation–based drug in single clones derived from glioma cells.

* indicates $p < 0.05$. Data are presented as the mean ± S.E.M.

Ⅳ. DISCUSSION

In previous study, it was shown that malignant gliomas can be reprogrammed into functional neurons that have an action potential, using a combination treatment of forskolin and CHIR99021 (GSK3 inhibitor) (in press). It is very exciting that the fate of gliomas can be changed diversely, into benign neurons or glial cells, depending on the various small molecule combinations.

In preliminary study, the conversion of malignant glioma cells to glial cells was tried, using small molecule compounds that have been widely used for glial differentiation. Unexpectedly, the malignant glioma cells were not changed to the glial-specific morphology in the presence of T3 and forskolin, which are known to induce glial differentiation.[16] After various experiments were performed, it was determined that the glioma can be transformed to the glial-specific morphology using only forskolin and rapamycin, without T3. It is thought that the characteristics and differentiation mechanism of malignant gliomas are not the same as those of glial precursor cells; however, gliomas are derived from glial precursor cells, and a significant portion of gliomas still strongly react with glial-precursor-cell-specific markers.[14,15] A previous study reported that mTOR inhibition prevents oligodendrocyte differentiation.[17] mTOR inhibition was shown to prevent the conversion of glial precursor cells into gliomas.[14] However, the role of mTOR inhibition in glioma reprogramming into benign glial cells remains unknown. These also indicates that the differentiation mechanism of malignant gliomas is not the same as those of glial precursor cells, and the synergistic effect of cAMP activation and mTOR inhibition plays a major role in glioma conversion into benign glial cells.

BET inhibitor was classified as an anticancer agent in clinical trials in the United States and Europe.[18,19] In this study, a BET (bromodomain and extra-terminal motif) inhibitor, I-BET151, was added to the media 7 days after induction of glial differentiation using a combination of forskolin and rapamycin. Treatment with I-BET151 helped maintain the glial-specific morphology, but also strongly inhibited cell proliferation (Fig. 2C~F, 4D~E). A previous study reported that the BET inhibitor accelerates the differentiation of mouse primary glial progenitors into oligodendrocytes.[20] However, this is the first study to report that I-BET151 affects the differentiation of a malignant glioma into benign glial cells.

It has been previously reported that the heterogeneity of glioblastomas affects their drug response, with each single clone responding differently.[21] In this study, it was observed that some cell populations did not convert to glial-like morphology after treatment of forskolin, rapamycin and I-BET151, and the most of its populations remained positive to Ki67. Thus, the gene expression profiling in single clones such as No. 4 or 8 clones was compared. In a microarray analysis, the malignancy or metastasis-related genes were highly expressed in No. 8 clone (Fig. 5E, F).[22-29]

In addition, *pdgfrb* and *sox2* were higher in No. 8 than in No. 4 (data not shown). It had been reported the expression and amplification of *Sox2* in glioma.[30] In brain tumors, *Sox2* expression is positively correlated with the malignancy grade.[31,32] It had been reported that *pdgfrb* expression is correlated with the metastatic behavior in cancer[33]. *pdgfrb* preferentially express in glioma stem cell and it's activation promotes glioma stem cell self-renewal.[34]

Especially, *mpz* and *plp1* are highly over-expressed in No. 8. It had been reported that *mpz* and *plp1* are closely related with myelin and oligodendroglioma.[35,36] Given that No. 8 clone showed good response to

forskolin, rapamycin and I-BET151, it indicates that the above gene lists may be specific marker to them. Future studies might need to investigate whether the expression level of above genes are implicated in glial induction through a combination treatment of these small molecules.

Moreover, it had been reported that *pdgfra*, *pdgfrb*, *pdgfrl*, *met*, *vegfa* and *colla1* implicated in cancer invasion and proliferation.[37,38] This result shows that the expression of above genes was down-regulated by treatment of small molecule (Fig. 3A), thus it indicates that the glial differentiation of gliomas may affect to benignancy.

Moreover, it is important to note that the small molecule composition strongly inhibited the proliferation of glioma cells, compared to standard anticancer drugs such as TMZ. Therefore, these differentiation-based anticancer drugs could potentially be used to target TMZ-resistant tumors. However, extensive research using patient-derived gliomas is required to confirm this hypothesis.

Ⅴ. CONCLUSION

These findings show the proof of concept that malignant gliomas, derived by mutations of glial precursor cells, can be converted to benign glial cells that show glial-specific characteristics. Moreover, the proliferation of malignant cells can be highly suppressed by the differentiation through the combination treatment of a cAMP activator, mTOR inhibitor, and BET inhibitor. Therefore, it is suggested that differentiation-based anticancer drugs may be the next generation of anticancer agents that act by converting malignant gliomas into benign glial cells.

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ABSTACT (IN KOREAN)

저분자화합물의 조합 처리를 이용한 악성 신경 교모세포의 양성 신경교세포 전환

<지도교수 하윤>

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김용보

중추신경계 종양 중에 가장 악성인 신경교모세포는 생존율이 매우 저조한 것으로 알려져 있다. 만약 신경교모세포가 신경교세포 전구체의 변이에 의해 유래되었다면, 신경교모세포의 상당부분이 신경교세포 전구체의 특이적 마커에 여전히 강한 양성반응을 보일 것이다.

따라서 신경교모세포가 내재적으로 보유하고 있는 신경교세포 전구체 관련 유전자 발현 조절을 통해, 신경교모세포를 본래 신경교세포 전구체가 최종분화 되고자 했던 신경교세포로 전환할 수 있는지를 조사하였다.

본 연구에서는 악성 신경교모세포를 별아교세포 혹은 희소돌기아교세포와 같은 양성의 신경교세포로 분화 유도하기 위하여, 세 개의 저분자화합물(cAMP 증강제와 mTOR 억제제 그리고 Bet 억제제)이 사용되었다. 저분자화합물에 의해 유도된 신경교모세포는 신경교세포의 특이적인 형태로 전환되었을 뿐 아니라 신경교세포 특이적 마커인 GFAP, CNP, RIP 에 각각 양성반응을 보였다. 마이크로어레이분석을 통한 유전자 발현 패턴의 변화를 살펴 본 결과, 분화 유도된 신경교모세포는 신경교세포에서

특이적으로 발현되는 유전자가 크게 증가된 반면, 악성 암세포에서 특이적으로 발현되는 유전자가 크게 감소하였다.

또한, 악성 신경교모세포에 저분자화합물을 처리하여 신경교세포로 분화 유도할 때, 악성 세포의 증식을 크게 억제할 수 있었다.

본 연구는 저분자화합물의 조합을 처리하였을 때, 악성 신경교모세포가 양성의 신경교세포로 전환될 수 있으며, 이에 따라 신경교모세포의 증식이 억제될 수 있음을 보여주었다. 따라서 이러한 저분자화합물의 구성(forskolin, rapamycin, I-BET 151)이 악성 신경교모세포를 양성의 신경교세포로 전환하여 치료하는 방식의 항암제로 개발될 수 있을 것으로 생각된다.

핵심되는말: 신경교모세포, forskolin, rapamycin, I-BET151, 신경교세포분화