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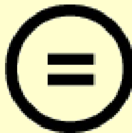

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의학석사 학위논문

The Study on Cancer invasion and Epithelial-Mesenchymal Transition by STAT3 and CXCR4 in Glioblastoma primary cells

교모세포종에서 STAT3와 CXCR4에 의한 암 침윤과 상피중간엽세포이행에 관한 연구

2013년 2월

서울대학교 대학원

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이 영 주

The Study on Cancer invasion and Epithelial-Mesenchymal Transition by STAT3 and CXCR4 in Glioblastoma primary cells

February, 2013

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ABSTRACT

The Study on Cancer invasion and Epithelial-Mesenchymal Transition by STAT3 and CXCR4 in Glioblastoma primary cells

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Glioblastoma is a highly invasive and aggressive brain malignancy. Despite significant improvements in early diagnosis and treatments of primary brain tumors, still many patients with malignant glioblastoma will survive less than one year after diagnosis from aggressive progression and recurrence. Although the exact causes or the

mechanism beneath are not clear yet but there is certainly an increase of interest in the correlation between progression of glioblastoma and of its STAT3 and CXCR4 expression.

STAT3 is associated with proliferation of glioblastoma stem cells. Recent study reported that CXCR4 is a chemokine receptor known to mediate glioma cell invasiveness. According to these reports, I could predict that STAT3 and CXCR4 are the important factors to not only aggressiveness and invasiveness but also survival period of patients in glioblastoma.

Thus this study aims to investigate the association between invasion, migration and Epithelial-Mesenchymal transition of glioblastoma with pY^{705}STAT3 and CXCR4 expression. The primary glioblastoma cells with high pY^{705}STAT3 level overexpress CXCR4, resulting in enhanced EMT and migration in vitro. CXCR4 silencing reduced the EMT and migration of primary glioblastoma cells. In contrast the primary GBL cells that have low pY^{705}STAT3 level hardly express CXCR4, resulting in reduced EMT and migration. Moreover, CXCR4 was regulated by STAT3 from the fact that STAT3 overexpression lead to increase level of CXCR4 expression in GBL cells. Even more interesting is the CXCR4 overexpression can up-regulate STAT3

expression in GBL cell and STAT3 directly interacts with CXCR4.

Taken together STAT3 derepressed glioblastoma expressed CXCR4 robustly and demonstrated a correlative relationship between expression levels of the CXCR4 and aggressiveness of the cells. My findings suggest that STAT3 related CXCR4 expression enhances EMT phenomenon and invasive potential of GBL cells. Therefore, STAT3 and CXCR4 expression status may be a useful potential therapeutic target in malignant brain tumor.

Keywords: Glioblastoma, STAT3, CXCR4, EMT, Cancer invasion

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LIST OF ABBREVIATION

GBL : Glioblastoma

STAT3 : Signal transducer and activator of transcription 3

pY^{705}STAT3 : Tyrosine 705 STAT3 phosphorylation

pS^{727}STAT3 : Serine 727 STAT3 phosphorylation

CXCR4 : C-X-C chemokine receptor type 4

EMT : Epithelial-Mesenchymal Transition

MRI : Magnetic resonance image

INTRODUCTION

Glioblastoma is the most common and aggressive malignant type of primary brain tumor in humans. It is also known as a grade IV astrocytoma according to the World Health Organization (WHO) classification. This type of tumor commonly occurs in adults between the ages of 45 to 70 and general treatment to remove or reduce size of glioblastoma [1-3]. Despite radio therapy after surgical removal and anticancer drugs administration of the whole body, many patients will survive less than one year after diagnosis [4, 5]. Generally wide excision, which involves removing additional normal tissue around the cancer, is the standard treatment for many types of cancer [6, 7]. Due to the nature of the brain, glioblastoma is adjacent to major functional region, impossible cases of wide excision are common. Temozolomide is an oral alkylating agent recommended treatment for newly-diagnosed glioblastoma and for recurrent high-grade gliomas [8]. In spite of these treatments, full recovery of glioblastoma is too hard caused by drug resistance and tumor recurrence. Therefore still urgently need for development of

new targeted drugs and combined therapy.

CXCR4, C-X-C chemokine receptor type 4 also known as CD184 and specifically binds CXCL12 [9, 10]. Their binding initiates diverse signaling pathways those lead to chemotaxis, cell survival, proliferation, up regulation of the intracellular calcium and participation in gene transcription [11]. CXCR4 is reported that there is no or low expressed in normal tissue but highly expressed in cancer [12].

The Epithelial-Mesenchymal Transition (EMT) is a phenomenon in normal embryonic development process. It means the process that cells loss the epithelial phenotype and change the mesenchymal phenotype which have high movement [13, 14]. If the process is proceeded irreversible, not only it brings about malfunction of heart, liver, kidney and vascular but also migration of malignant tumor [15]. EMT begins from the dissociation of the intracellular junctions, as a consequence of down regulation of adhesion molecules such as E-cadherin, claudins, occlaudins, zo-1 also loss of apical-basal polarity. Thus arise cytoskeleton reorganization and express alpha-smooth muscle actin and increase migratory capacity is due to cells possess Front-back polarity. In the last stage of the EMT, basement

membrane rise the degrade capacity and cells have fibrotic stromal invade capacity with growth of MMP expression. Therefore, mesenchymal markers like N-cadherin, Collagen, Fibronectin, Snail, Vimentin and Integrins are up regulated [14, 16].

As already well known, STAT3 is highly expressed in glioblastoma and required for proliferation and maintenance of multipotency in glioblastoma stem cells [17]. Also recently, the study on STAT3 regulation in glioblastoma pathogenesis and STAT3 targeting are performed [18, 19]. STAT3 inhibition is up regulated drug efficacy in temozolomide resistance gliobalstoma [20]. Taken together, STAT3 expression is a pivotal factor for glioblastoma progression.

The study on a significance of CXCR4 expression in cancer has been reported. CXCR4 is not only highly expressed in glioblastoma [21], but also mediates invasiveness and proliferation [22]. Especially, the report showing CXCR4 is essential for maintenance of stemness in drug resistance non-small cell lung cancer cells suggests that CXCR4 can regulate phospho tyrosine STAT3 [23].

According to reports above, I can predict that STAT3 and CXCR4 are important factors for tumor aggressiveness and invasiveness in

glioblastoma. Furthermore they play a crucial role in diagnosis and survival of patients. So here I show that, the effect of 2 factors on diagnosis and correlation between STAT3 and CXCR4 in human primary cells from glioblastoma patients.

MATERIALS AND METHODS

Human Primary cell culture

Human malignant glioblastoma, GBL #12, 14, 15, 28, 30, 34, 37 cells were obtained in collaboration with Dr. Sun Ha Paek at Seoul National University Hospital (Seoul, Korea). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% of heat- inactivated fetal bovine serum and antibiotics at 37℃ with 5% CO_2 .

Transfection experiments

For STAT3 and CXCR4 knock-down, small interfering RNA (siRNA) was used. STAT3 and CXCR4 siRNAs (Qiagen, Valencia, CA, USA) were transfected into cells using HiPerFect (Qiagen) according to the manufacturer’s instructions. To generate a plasmid encoding the STAT3 and CXCR4 gene, the gene was amplified by

polymerase chain reaction (PCR) and cloned into the pcDNA3.0 vector (Invitrogen, Carlsbad, CA, USA). The STAT3 or CXCR4 construct or an empty (control) plasmid was transfected into cells using Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol.

Immunoblotting and immunoprecipitation

7 kinds of GBL cells, GBL 30 silenced with STAT3 or CXCR4 and GBL 15 transfected with STAT3 or CXCR4 plasmid, were harvested in a lysis solution (Santa Cruz Biotechnology, Santa Cruz, CA, USA) containing a protease inhibitor cocktail (Roche) and a phosphatase inhibitor (Roche). After incubation for 20 min on ice, insoluble debris were removed by centrifugation for 20 min at 4℃. Total protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (GE Healthcare, Pittsburgh, PA, USA). The membranes were then probed with antibodies towards α-tubulin (Thermo Fisher Scientific, Fremont, CA, USA), pY^{705}STAT3 (Cell Signaling

Technology, Danvers, MA, USA), and CXCR4 (Abcam, Cambridge, UK) and visualized using the SuperSignal West Femto Chemiluminescent Substrate (Thermo Fisher Scientific). For immunoprecipitation, lysates (1 mg proteins) were incubated with 1 μg of antibodies and further incubated with 20 μl of protein-A/G beads for 4 h. Precipitated immuno-complexes were eluted with a SDS buffer and then subjected to immunoblotting.

Immunofluorescence staining and confocal microscopic imaging

GBL 12, 14, 15 and 30 cells were grown on 35-mm lysine-coated, glass-bottomed culture dishes (MatTek, Ashland, MA, USA), followed by 10 min fixation with 3.7% paraformadehyde. To permeabilize cell membranes, cells were treated with 0.1% Triton X-100 at 37℃ for 30 minutes. After blocking nonspecific bindings in PBS containing 0.1% Triton X-100 and 3% Bovine Serum Albumin, cells were incubated with primary antibodies overnight at 4℃. Cells were then incubated with secondary antibodies (1:500) conjugated with DyLight 488 (Vector Laboratory) or Alexa 588 (Invitrogen) for

2 hours. Primary antibodies were used against CXCR4 (1:100; abcam, Cambridge, MA) and ZO-1 (1:200; Cell Signaling Technology). Immunofluorescence F-actin staining was done using an Alexa Fluor 633 phalloidin (Molecular Probes, Invitrogen, Carlsbad, CA). Briefly, cells were preincubated with PBS containing 1% bovine serum albumin, and incubated with Alexa Fluor 633 phalloidin (2-3 units) for 20 minutes. Cell nuclei were counter stained with 1g/ml of DAPI (Sigma). The slides were imaged using a Zeiss Axiovert 200 fluorescence microscope with LSM 510 META system.

Quantitative reverse-transcription-polymerase chain reaction (PCR) assay

Total RNA was isolated from 7 types of GBL cells. The cDNA was synthesized using a QuantiTech Reverse Transcription Kit (Qiagen) and was then mixed with QuantiFast SYBR Green PCR master mix (Qiagen) and specific primers for CXCR4, TWIST1 and GAPDH obtained from Qiagen. Quantitative reverse-transcription

PCR (qRT-PCR) was performed with an Applied Biosystems 7300 Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). The raw data were analyzed by comparative Ct quantification.

Wound healing assay

Cells were seeded onto a 6 well ($5x10^5$) plate, incubated with 90% of confluence. The plate is scratched using pipette tips, washed with PBS, transfected with STAT3 and CXCR4 plasmid DNA or siRNA. Control images are taken at 0h, comparison images are taken after 24h incubation.

Cell invasion assay

Invasiveness was assessed using a Boydenchamber system (Neuro Probe, Gaithersburg, MD, USA) with a polycarbonate membrane having a pore size of 8 μm separating the two chambers. The upper chamber was coated on ice with 100 μl of growth factor-reduced

Matrigel (BD Biosciences, San Jose, CA, USA) containing 0.5 mg/ml protein and was then incubated at 37˚C for at least 4-5 h to allow gelling. Cells (1×10^4), including GBL 15 and GBL 15 transfected with STAT3 and CXCR4 plasmid DNA or GBL 30 and GBL 30 transfected with STAT3 and CXCR4 siRNA, in 100 μl of serum-free Dulbecco's modified Eagle's medium (DMEM) were loaded into the upper chamber. The lower chamber was filled with 600 μl of DMEM containing 10% fetal bovine serum and 5 mg/ml fibronectin. The chambers were incubated at 37˚C for 24 h in an atmosphere 95% air and 5% CO_2. The upper medium was then removed, the chamber disassembled, and the membranes were rinsed in PBS and fixed. The membranes were subsequently stained with Diff-Quick solution (Sysmex Corp. Kobe, Japan) and then washed twice. Cells attached to the bottom of the membrane were photographed at ×10 magnification

Statistical analysis

Comparisons of multiple groups were done by analysis of variance (ANOVA).

RESULTS

pY^{705}STAT3 is differently expressed in Glioblastoma primary cells

7 types of primary cells were obtained from patients with grade Ⅳ glioblastoma. To characterize the glioblastoma, pY^{705}STAT3 expression in protein level was investigated by western blotting in unknown about any information of patients. Although all types of cells are grade Ⅳ glioblastoma, each of these cells shows the different rate of cell growth and different expression of pY^{705}STAT3 (Fig. 1A). To visualize the contrast of pY^{705}STAT3 levels, the ratio of pY^{705}STAT3 to STAT3 expression was indicated on a graphs (Fig. 1B). Next, 4 kinds of GBL primary cells that are 12, 14, 15, 30 were selected the depending on pY^{705}STAT3 expression and examined the morphology of the cells. In GBL 12, 15 primary cells that have low levels of pY^{705}STAT3 expression are blunt and spherical also slower growth rate. By contrast, GBL 14, 30 primary cells that have high levels of pY^{705}STAT3 expression are sharp and long with shorter

doubling time than GBL 12, 15 primary cells (Fig. 1C). GBL 15 and 30 were selected from the two different types for simpler and easier handling.

Prognosis of glioblastoma patients correlates with phosphorylated STAT3 expression

MRI (magnetic resonance image) of GBL 15 and 30 corresponsive patients were analyzed. A 39-year-old female with brain tumor on the left anterior temporal lobe diagnosed grade IV glioblastoma. Though passing of time, the tumor did not spread surrounding region and the patients survived over one year after diagnosis (Fig. 2A). A 48-year-old female with brain tumor on the left frontal area diagnosed grade IV glioblastoma. The tumor spread very fast to other area as time goes by. A cerebral ventricle collapsed by tumor 7 months after diagnosis (Fig. 2B). Interestingly the pY^{705}STAT3 expression level and patients' prognosis in the previously western blot data showed exhibited correlation (Fig. 1A). Therefore even if the patients are in the same grade of glioblastoma, exhibited

different aggressiveness depends on pY^{705}STAT3 expression.

CXCR4 expression correlates with phosphorylated STAT3

CXCR4, a chemokine receptor is significantly overexpressed in high-grade human gliomas and related to enhanced invasiveness of cancer cells [21, 22]. Therefore, it was investigated the level of CXCR4 proteins in GBL 15 comparing with GBL 30 tissue. The results of CXCR4 immunostaining showed that GBL 30 expressed CXCR4 more than GBL 15 (Fig. 3A). In addition the expression of CXCR4 in 7 types of GBL primary cells was checked. CXCR4 expression at the mRNA and protein levels was significantly higher in GBL 14, 28, 30, 37 cells that have high levels of pY^{705}STAT3 expression (Fig. 3B and C). To visualize these results, Correlation Analysis between relative fold changes of pY^{705}STAT3 expression in figure 1B and CXCR4 mRNA expression of 7 types of GBL primary cells were performed. Interestingly, CXCR4 expression is significantly correlated with expression of pY^{705}STAT3 (Fig. 3D).

pY^{705}STAT3 expression enhances invasiveness and EMT in Glioblastoma

The morphological differences between GBL 15 and 30 cells were already confirmed. For these reason, it seems that GBL 30 cell, highly expressed in STAT3 was related in Epithelial-Mesenchymal Transition (EMT). Therefore various genes include EMT markers at the mRNA and/or protein levels in cells with high and low pY^{705}STAT3 expression were checked. Integrin αV (a msenchymal marker), MMP2 (is involved in cancer cell migration) and VEGF (a major contributor in angiogenesis) were highly expressed, but Zo-1 (a tight junction protein) was lowly expressed in GBL 30 cell that have high levels of pY^{705}STAT3. In addition cancer stem cell markers such as Nestin, Sox2, Nanog and CD133 were highly expressed in GBL 30 cell (Fig. 4A and B). It was performed immunofluorescence in GBL 15 and 30 cells. GBL 30 cells showed many long F-actin stress fibers, which were not found in GBL 15 cells (Fig. 4C). In addition, ZO-1, is lost during EMT [24], was dramatically reduced in GBL 30 cells (Fig. 4D). Taken together, these results suggest that pY^{705}STAT3 expression is strong in

aggressive and cancer stem like GBL cells, resulting in enhanced invasiveness and EMT in vitro and possibly enhanced metastasis in vivo.

pY^{705}STAT3 and CXCR4 can induce Epithelial-Mesenchymal Transition

As mentioned earlier, pY^{705}STAT3 expression is related to invasiveness, EMT and metastasis in glioblastoma. To investigate the effects of STAT3 and CXCR4 on EMT, the expression of EMT markers after STAT3 and CXCR4 overexpression or STAT3 and CXCR4 knockdown were checked. CXCR4 and STAT3 overexpression by STAT3 and CXCR4 plasmid DNA reduces Zo-1 and induces pFAK, integrin αV, N-cadherin, Twist1, Fibronectin and VEGF at the protein and mRNA level (Fig. 5A, B and C). On the other hand, STAT3 knockdown by siRNA induces Zo-1 and reduces CXCR4, pFAK at the protein level. In addition, not only integrin αV, N-cadherin but also, Fibronectin was reduced at the mRNA level in STAT3 knock down GBL 30 cell (Fig. 5D and E). Knockdown of

CXCR4 by siRNA decreases expression of phospho FAK (Focal adhesion kinase-1) and increases Zo-1. Furthermore integrin αV, N-cadherin, Twist1 and Fibronectin were down regulated at the mRNA level (Fig. 5F and G).

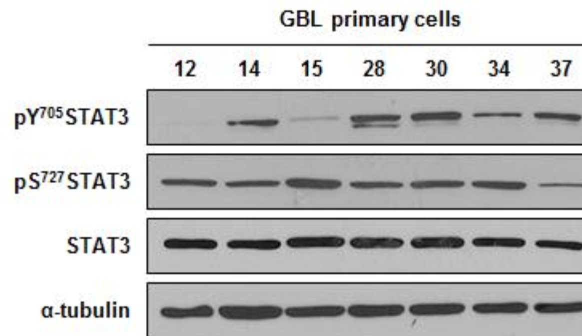
pY^{705}STAT3 and CXCR4 can promote cell migration and invasion

To confirm that the CXCR4 and pY^{705}STAT3 are involved in cell migration, wound healing assay and matrigel invasion assay were done. Control image was taken at 0h. The GBL 15 cell resulted promotion in motility upon transfection with plasmid DNA (Fig. 6A) and GBL 30 cells resulted suppression in motility upon knock down with siRNA (Fig. 6B). Matrigel invasion assay was carried to measure the alteration of invasiveness. Cell invasiveness of both GBL 15 cells were transfected with STAT3 or CXCR4 was significantly increased (Fig. 6C). Also cell invasiveness of both GBL 30 cells were transfected with siSTAT3 and siCXCR4 were dramatically reduced (Fig. 6D).

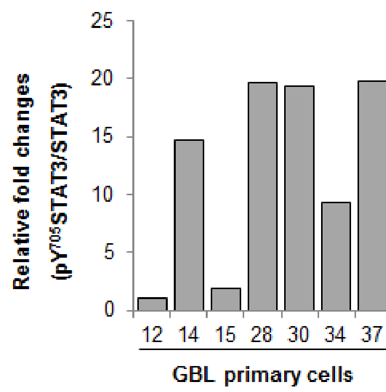
STAT3 interacts with CXCR4 in glioblastoma primary cell

Previously mentioned, pY^{705}STAT3 and CXCR4 expression are important factors for tumor aggressiveness. To check the connection between STAT3 and CXCR4, GBL 30 cell was transfected with STAT3 plasmid DNA. STAT3 overexpression leads to up regulation of CXCR4 in GBL 30 cell (Fig 7A). The interaction between STAT3 and CXCR4 was examined by co-immunoprecipitation. pY^{705}STAT3 was found to co-precipitate with CXCR4 (Fig 7B).

(Fig. 1A)



(Fig. 1B)



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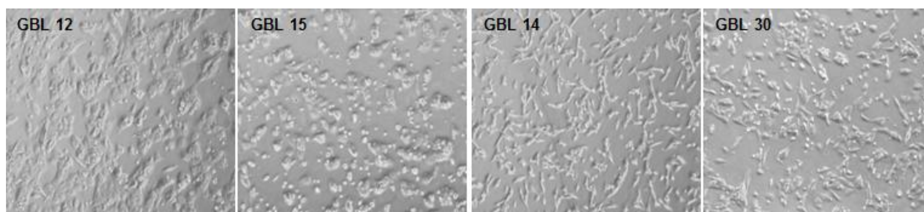
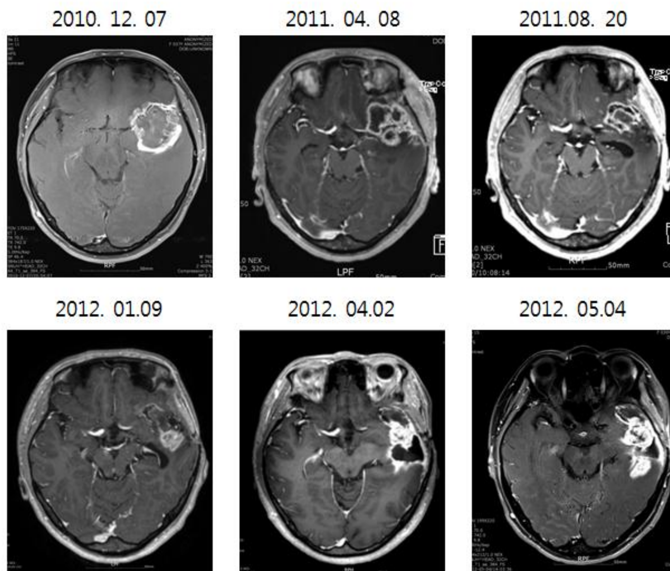


Figure 1. Character comparison on pY^{705}STAT3 expression in glioblastoma primary cells. (A) Whole extracts prepared from glioblastoma primary cells were subjected to western blotting analysis with different antibodies as indicated. (B) Quantitation of STAT3 phosphorylation data. Amount of STAT3 phosphorylation was quantitated by an image analyzer. Data represent the mean±SEM of the relative ratio to total STAT3 expression of three different experiments. $*p<0.05$ (C) A representative phase-contrast photograph of GBL 12, 15, 14, 30 cells. Magnification, 50×

(Fig. 2A)



(Fig. 2B)

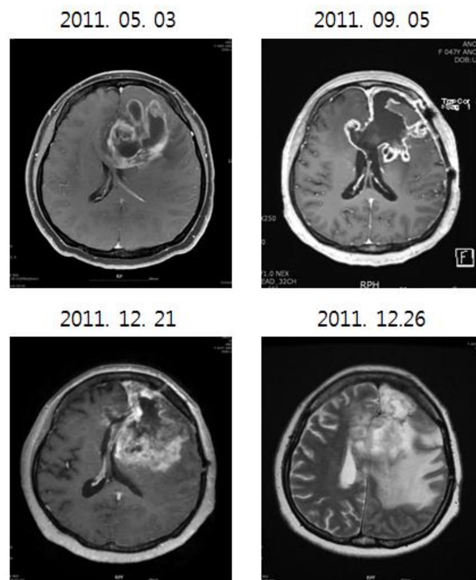
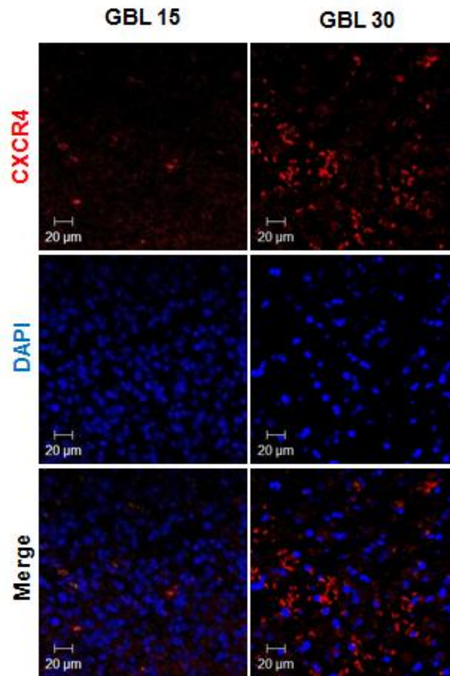
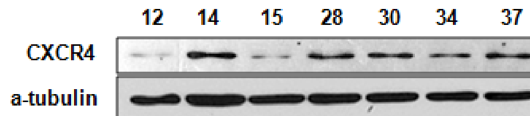


Figure 2. The comparison of prognosis in patients with glioblastoma according to the difference of phosphorylated STAT3 expression. (A) 39-year-old female with a glioblastoma on the left anterior temporal lobe. The tumor is mild and not severe that was applicable to GBL 15 cell. (B) 48-year-old female with a glioblastoma on the left frontal area. The tumor is aggressive and severe that was applicable to GBL 30 cell.

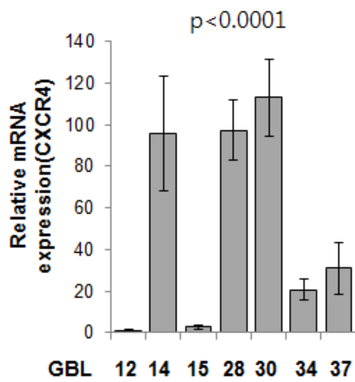
(Fig. 3A)



(Fig. 3B)



(Fig. 3C)



(Fig. 3D)

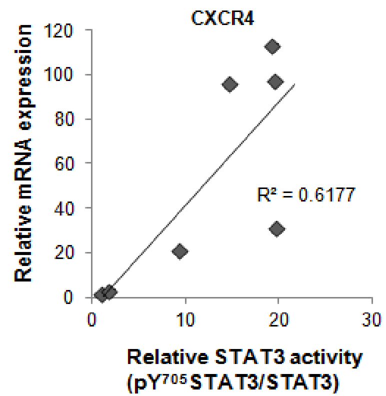
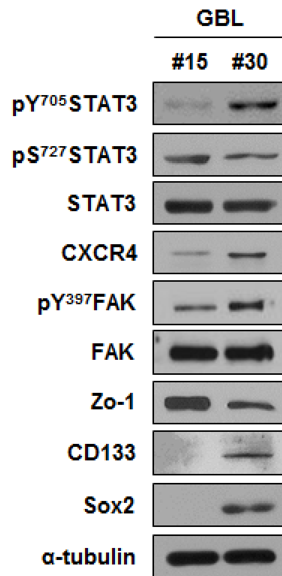
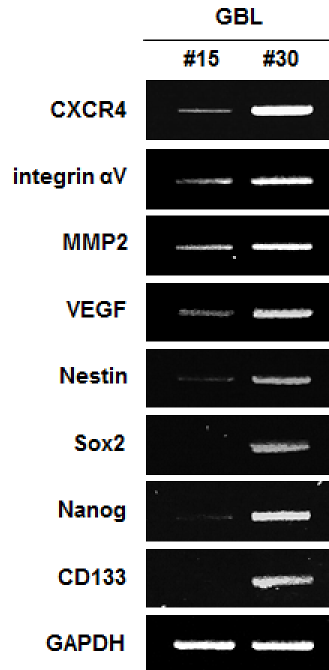


Figure 3. CXCR4 expression in GBL primary cells. (A) Tissues from 2 types of glioblastoma patients were stained with anti-CXCR4 (red) antibodies, and nuclei were stained with DAPI (blue). Images were taken under a confocal microscope using a ×40 objective. (B) CXCR4 protein levels in 7 types of GBL primary cells were measured by immunoblotting. (C) The transcription level of CXCR4 in 7 types of GBL cells was analyzed by quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The qRT-PCR data were analyzed by comparative Ct quantification. Data were presented as the mean±SEM (n=3). p-Values were obtained using ANOVA. (P value<0.0001) (D) Correlation analysis of mRNA expression between CXCR4 and relative $pY^{705}STAT3$ activity. Relative $pY^{705}STAT3$ activity was significantly correlated to CXCR4 mRNA. The scattered plots were summarized as relative expression levels of $pY^{705}STAT3$ activity associated with relative CXCR4 mRNA expression.

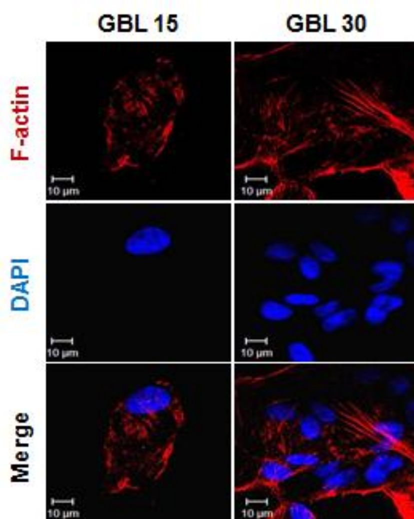
(Fig. 4A)



(Fig. 4B)



(Fig. 4C)



(Fig. 4D)

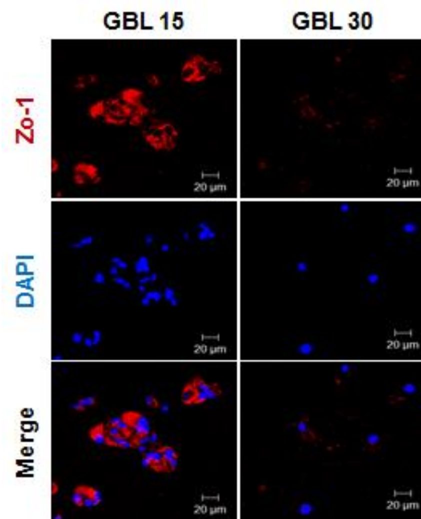
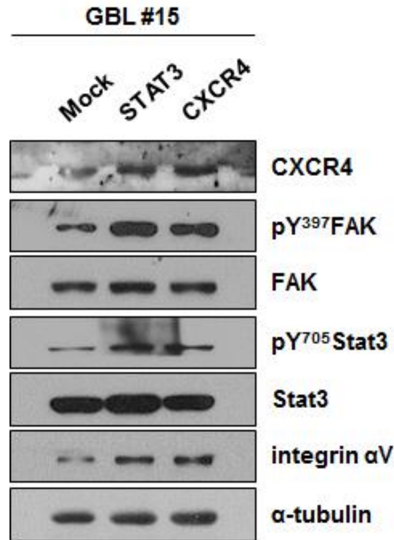
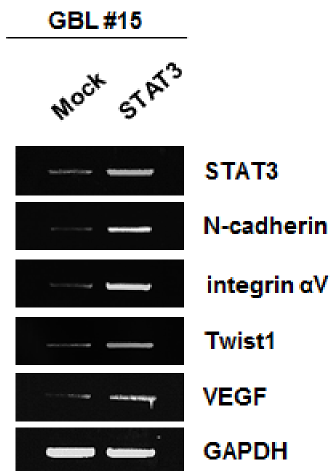


Figure 4. pY^{705}STAT3 expression enhances aggressiveness in glioblastoma. (A) Equal amounts of proteins from GBL 15 and 30 cells were subjected to western blotting using the indicated antibodies. (B) Total RNA was then extracted and subjected to RT-PCR analysis using primers. PCR products were resolved by electrophoresis on 2% agarose gels. (C) Immnofluorescence staining for F-actin (red) was performed, and all nuclei were stained with 4',6-diamidino-2-phenylindole (blue). Scale bars=10μm. (D) 2 types of glioblastoma primary cells were examined for Zo-1 (red) levels by immunofluorescence. DAPI, 4',6-diamidino-2- phenylindole. Scale bars=20μm

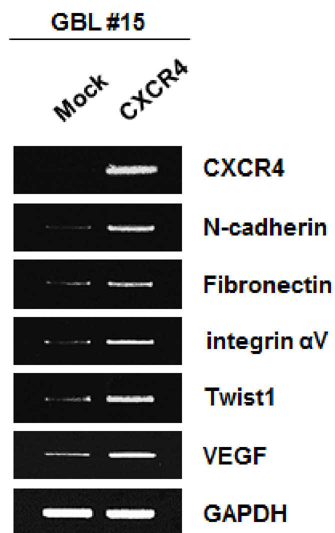
(Fig. 5A)



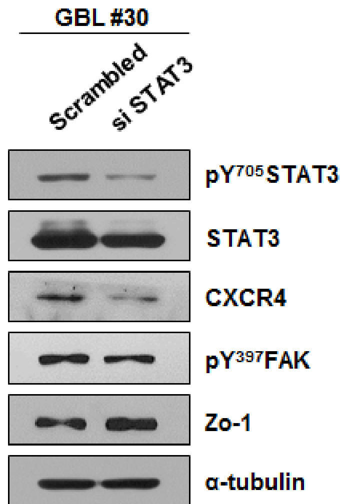
(Fig. 5B)



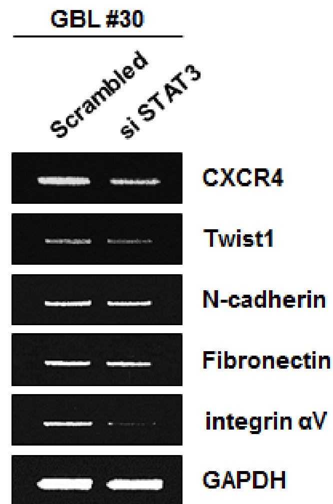
(Fig. 5C)



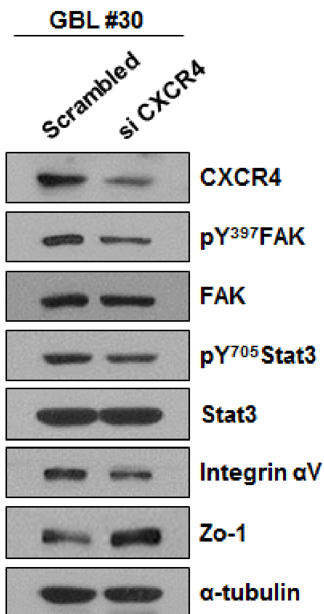
(Fig. 5D)



(Fig. 5E)



(Fig. 5F)



(Fig. 5G)

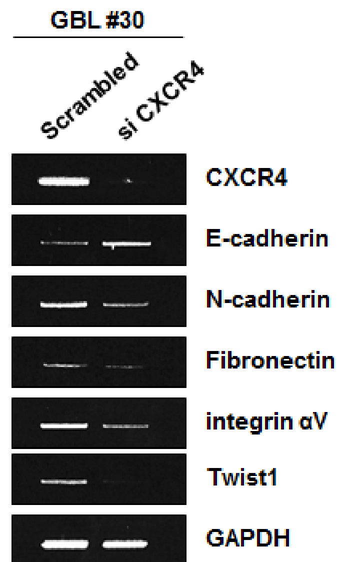
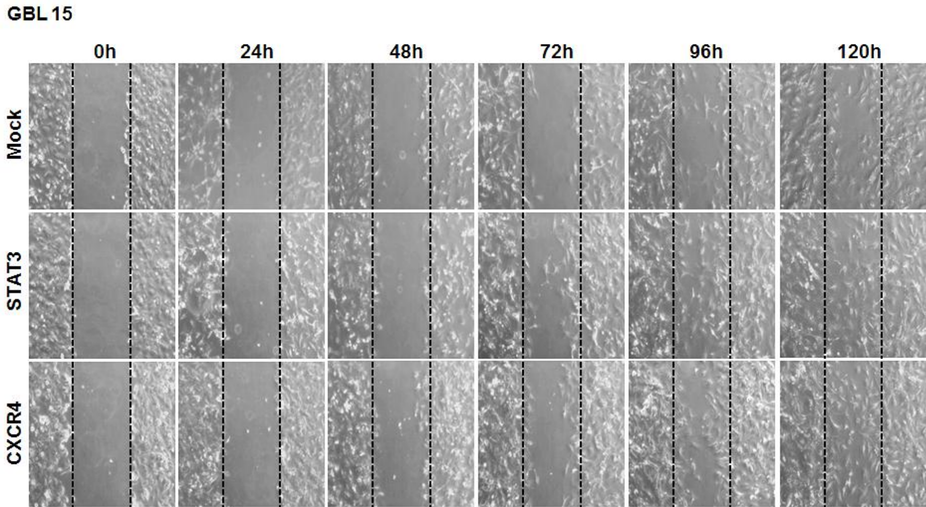
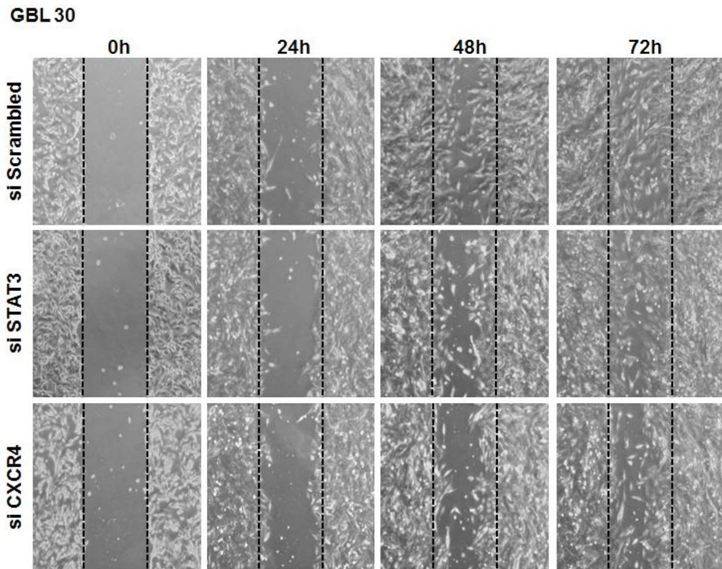


Figure 5. STAT3 and CXCR4 can induce EMT in vitro. (A) Representative immunoblots show CXCR4 and STAT3 protein levels in GBL 15 cells transfected with a plasmid encoding STAT3, CXCR4 gene or the empty vector pcDNA3.0 (as a control) at 48h after transfection. (B, C) RT-PCR data show CXCR4, STAT3 and number of EMT related gene mRNA levels in GBL 15 cells transfected with a plasmid encoding STAT3 gene or the empty vector pcDNA3.0 (as a control) at 48h after transfection. (D, E) STAT3 siRNA (si-STAT3, 20nM) or control siRNA (Control, 20nM) was transfected in to GBL 30 cells. Total RNA and protein were isolated from cells at 48h after transfection. The expression of STAT3 in the cells was analyzed by immunoblotting and RT-PCR. (F, G) CXCR4 siRNA (si-CXCR4, 20nM) or control siRNA (Control, 20nM) was transfected in to GBL 30 cells. Total RNA and protein were isolated from cells at 48h after transfection. The expression of CXCR4 in the cells was analyzed by immunoblotting and RT-PCR.

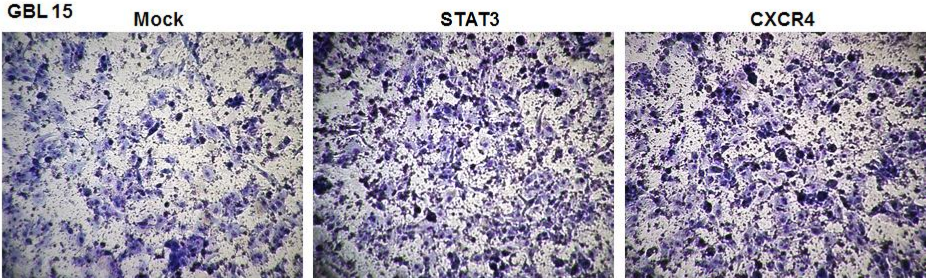
(Fig. 6A)



(Fig. 6B)



(Fig. 6C)



(Fig. 6D)

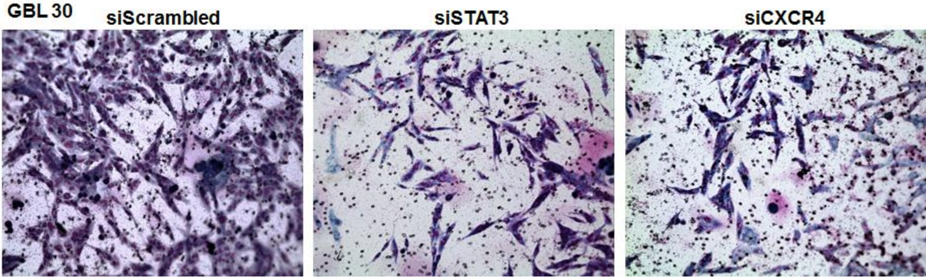
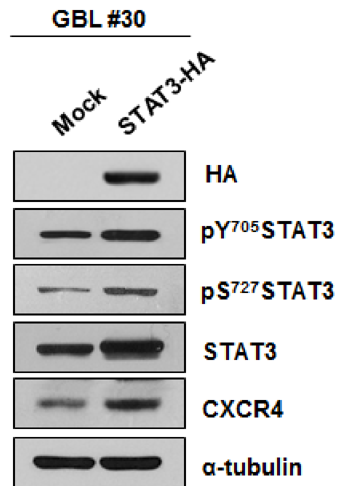


Figure 6. STAT3 and CXCR4 promote cell migration in vitro. (A, B) Representative Wound healing migration assay photographs. Cell migration of GBL primary cells in which STAT3 and CXCR4 had been overexpressed or silenced was assessed to 72h or over after transfection. (C, D) Representative Matrigel Invasion assay photographs. The invasive behavior of GBL primary cells in which STAT3 and CXCR4 had been overexpressed or silenced was assessed at 24h after transfection.

(Fig. 7A)



(Fig. 7B)

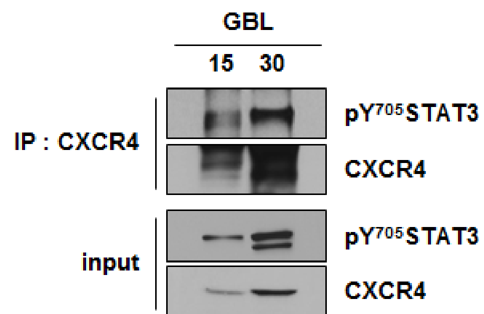


Figure 7. STAT3 interacts with CXCR4. (A) Representative immunoblots show STAT3 and CXCR4 protein levels in GBL 15 cells transfected with a plasmid encoding STAT3 gene or the empty vector pcDNA3.0 (as a control) at 48h after transfection. (B) GBL 15 cells were transfected with STAT3 and CXCR4, and cell extracts were immunoprecipitated with anti-CXCR4, and co-precipitated Flag-STAT3 was analyzed using anti- pY^{705}STAT3. GBL 30 cell's whole lysates were immunoprecipitated with anti-CXCR4.

DISCUSSION

In this study, I demonstrated that $pY^{705}STAT3$ is differently expressed in the many types of grade Ⅳ Glioblastoma primary cells, which determined morphological and aggressive character of GBL cells (Figure 1). The expression of $pY^{705}STAT3$ in Glioblastoma primary cells is correlated with prognosis of patients (Figure 2). CXCR4 expression is also correlated with $pY^{705}STAT3$, as verified by qRT-PCR, Immunoblotting and Immunofluorescence (Figure 3). GBL 30 cell, represents high $pY^{705}STAT3$ expression, is more aggressive and metastatic than GBL 15 cell, represents low $pY^{705}STAT3$ expression (Figure 4). According to a recent report, up-regulated CXCR4 is an important maintenance factor of stemness in drug-resistant non-small cell lung cancer cells [23]. As it is also identified that CXCR4 mediated tumor invasion [25] and up-regulated by VEGF in glioma [26, 27]. I scrutinized the roles of $pY^{705}STAT3$ and CXCR4 for Epithelial-mesenchymal Transition (EMT) in glioblastoma (Figure 5). Moreover $pY^{705}STAT3$ and CXCR4 can induce migration and invasiveness (Figure 6).

In agreement with my data, STAT3 and CXCR4 overexpressed GBL cell result in increased EMT and tumor migration and invasiveness. I therefore suggest that STAT3 or CXCR4 dependent migratory and invasive potential have a decisive effect on lifespan of patients with glioblastoma. However, the definite mechanism of the interaction between STAT3 and CXCR4 still seems to be a little ambiguous.

In conclusion, this study provides insight about the functional significance of increased STAT3 and CXCR4 expression in GBL cells. Overexpression of CXCR4 in GBL cells is essential for EMT, migration and invasion suggesting that STAT3 and CXCR4 are not only prognostic markers, but also functional molecules in brain cancer cells. In addition, STAT3 and CXCR4 expression are dependent on each other. Although further study is needed to elucidate the mechanisms of STAT3-CXCR4, determining whether STAT3 acts on CXCR4 or otherwise in glioblastoma, this study would be of value.

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국문 초록

교모세포종에서 STAT3와 CXCR4에 의한 암 침윤과 상피중간엽세포이행에 관한 연구

이 영 주

의과학 전공

의과학과

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교모세포종 (glioblaastoma) 은 매우 침습적이며 빠르게 증식하는 악성의 뇌종양이다. 교모세포종의 조기 진단과 치료에 있어 괄목할만한 발전을 이루었음에도 불구하고, 종양의 급격한 증식과 재발 등의 이유로 많은 환자들은 진단 이후 1년 이내에 사망하고 있다. 그러나 이러한 요인들에 관한 정확한 원인과 메커니즘이

밝혀지지 않은 가운데, 교모세포종에서의 STAT3 (Signal Transducer and activator of transcription 3) 와 CXCR4 (C-X-C chemokine receptor type 4) 발현에 대한 관심이 증대되고 있다.

STAT3는 대부분의 종양에 활성화되어 있다고 알려진 전사인자로서 교모세포종 줄기세포의 증식과 연관성이 밝혀진 바 있으며, CXCR4는 chemokine receptor로 신경 교종세포의 침윤성을 매개한다고 보고된 바 있다. 이러한 단백질들의 발현은 세포의 이동현상인 상피중간엽세포이행 (Epithelial-Mesenchymal Transition) 과도 밀접한 관련성이 있다. 따라서 악성 교모세포종에서 STAT3와 CXCR4는 모두 EMT 현상을 통한 종양의 증식과 침윤에 중요한 인자로 작용할 수 있고, 환자의 예후와 생존기간에 필수적인 역할을 한다고 예측 할 수 있다.

그러므로 본 연구에서는 교모세포종의 상피중간엽세포이행에 있어 pY^{705}STAT3와 CXCR4의 발현이 가지는 연관성을 밝히고자 하였다. pY^{705}STAT3의 높은 발현을 가지는 교모세포종 1차 배양세포는 CXCR4를 높게 발현하였으며, 상피중간엽세포이행과 침윤이 증가된 양상을 보였다. pY^{705}STAT3와 CXCR4 발현의 중요성을 증명하기 위해 CXCR4 발현을 억제시켰고, 상피중간엽세포이행과

침윤이 감소되는 양상을 확인 할 수 있었다. 반면 pY^{705}STAT3의 낮은 발현을 가지는 교모세포종 1차 배양세포에 CXCR4를 과발현 시킴으로써 상피중간엽세포이행과 침윤이 증가 됨을 확인 할 수 있었다. 흥미롭게도, STAT3를 과발현 시켰을 때 CXCR4의 발현이 증가 될 뿐 아니라, CXCR4를 과발현 시켰을 때에도 STAT3의 발현이 증가 되었다. 더욱이 STAT3와 CXCR4는 서로 결합하고 있음을 확인할 수 있었고, 이는 두 단백질이 상호작용을 통해 서로를 조절한다고 예측 할 수 있다.

이러한 결과들을 종합하면, STAT3가 높게 발현 되어있는 교모세포종은 CXCR4를 강하게 발현하며, 이들의 발현 정도는 암세포의 침윤성와 밀접한 상관관계가 있음을 알 수 있었다. 연구 결과를 기반으로 STAT3와 CXCR4의 높은 발현은 상피중간엽세포이행 현상을 통해 침윤능력을 증가시킨다는 결과에 도달할 수 있었다. 그러므로 STAT3와 CXCR4는 악성의 뇌 암에서 중요한 치료적 타깃이 될 수 있을 것이다.

주요어 : 교모세포종, STAT3, CXCR4, 상피중간엽세포이행, 침윤

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