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The effect of redox factor-1 on the Cancer progression

조선대학교 대학원

생물신소재학과

정 성 미

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Redox factor-1이 암진행에 미치는 영향

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이 논문을 이학석사학위신청 논문으로 제출함.

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The effect of redox factor-1 on the Cancer progression

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ABSTRACT

APE/Ref-1 has been known the multifunctional protein which is involved in redox signaling, DNA repair, apoptosis and cell proliferation. In the previous studies, APE1 or JAG1 are high expressed in a variety of cancer. However, their roles of cancer are not clear, yet. Here, for the first time, I'd like to identify the function and the correlation between APE/Ref-1 and JAG1 in cancer. To investigate whether APE/Ref-1 controlled JAG1, I checked JAG1 expression in both APE/Ref-1-overexpressed or JAGGED1-downregulated cell line. I found that APE/Ref-1 regulated JAG1 expression. Recently, it is reported that cell proliferation is partially dependent on JAG1/Notch regulation. So, I investigated the correlation among APE/Ref-1, JAG1/Notch1 and Hes1. In APE/Ref-1 overexpressing cells, JAG1 and Notch1 activities were increased. Also, in JAG1-downregulated cells, their activities were reduced. Furthermore, *in vitro* cell migration is dependent on both of APE/Ref-1 and JAG1. In conclusion, APE/Ref-1 may regulate cell proliferation and migration through JAG1/Notch1 downstream effector, Hes1. I suggest that these finding may be contributed to provide new insight to the develop new anticancer therapies.

I. INTRODUCTION

Gliomas are the most common primary brain tumors and confer a grave prognosis. Standard therapies, such as chemotherapy, surgery, and radiation, have had limited success in treating patients with high-grade gliomas. It is hoped that a greater understanding of the molecular pathways involved in glioma cell proliferation and survival will lead to more effective targeted therapies.

Ape1/Ref-1 is a multifunctional protein that is not only responsible for the repair of AP sites but also stimulates the DNA-binding activity of the AP-1 family of transcription factors via a redox-dependent mechanism (1, 2). This effect is mediated via the reduction of a conserved cysteine residue located at the DNA-binding domains of c-fos and c-jun (3). Ape1/Ref-1 is also capable of modulating or activating other classes of transcription factors, including NF-κB, p53, Egr-1, c-Myb, HLF, and Pax-8, via a similar reducing action (4). The ability of Ape1/Ref-1 to activate the transcription factors involved in the cellular response to various stresses suggests that Ape1/Ref-1 may play an important role in various cellular processes. Studies have reported elevated Ape1/Ref-1 levels or altered subcellular localization in various types of cancers, such as epithelial ovarian cancers, cervical cancers, prostate cell tumors, melanoma, gliomas, rhabdomyosarcoma, and germ cell tumors, which are associated with tumor resistance and progression (5-11). In addition, alterations in Ape1/Ref-1 expression and mutations in the APE1 gene have been detected in patients with a variety of neurodegenerative diseases (12-14). Thus, APE1 has been implicated in tumor progression, and APE1 dysfunction may contribute to development of neurodegenerative disease. However, the molecular mechanisms underlying these effects are unclear.

Notch signaling is involved in cell proliferation and apoptosis, which affects the development and function of many organs. Four Notch receptors (Notch1-Notch4) and five DSL (named for Delta and Serrate from Drosophila and Lag-2 from C. elegans) ligands [Jagged-1, Jagged-2, delta-like1 (Dll-1), Dll-3, and Dll-4] have been described in mammals (15, 16). Activation of Notch signaling is

mediated by interactions of bordering cells via cell-to-cell contact of the membrane-associated Notch receptor and ligand. After ligand binding, 2 enzymatic cleavages occur to liberate the Notch intracellular domain (NICD) from the plasma membrane. The NICD, also known as ICN, translocates into the nucleus and binds to members of the CSL transcription factor family, which are thought to mediate most of the downstream effects of Notch signaling. Following NICD binding, the CSL family member CBF-1/RBP-JK, normally part of a corepressor complex with histone deacetylase 1, becomes a transcriptional activator. Downstream targets of CBF-1 include a large family of b helix loop helix(bHLH) transcription factors known as the hairy/enhancer of split(HES) genes. Alteration of these functions in the adult has been associated with various types of cancer in which Notch may act as an oncogene with certain exception (17, 18).

The aim of study was to investigate the role of APE through JAG/Notch signaling in glioma cell progression including proliferation, survival and migration. I examined the effect of small interfering RNA (siRNA)-mediated knockdown of APE1 on glioma cells and compared it with that of knockdown of JAG1. These findings may be significant for understanding of APE/JAG1/Notch1 signaling in cancer progression.

II. MATERIALS AND METHODS

1. Cell culture

Glioma cell lines U87MG, U373MG, M059K and M059J were all acquired from American Type Culture Collection (ATCC, Rockville, MD, USA).. M059K and M059J cells were grown in Dulbecco's modified Eagle's medium, Nutrient Mixture F-12 (D-MEM F-12) (Gibco, Grand Island, NY, USA) containing 10% FBS and 1% penicillin, respectively. The U373MG and U87MG cells were maintained in RPMI 1640 (Gibco) supplemented with 10% FBS and 1% penicillin. GM00637 (human fibroblast) cell was cultured in Eagle's minimum essential medium containing 10% fetal bovine serum (FBS) and 1% penicillin. All cells were maintained in cell-specific media at 37 °C in a humidified atmosphere of 5% CO_2.

2. siRNA knockdown of Ape1/Ref-1 and JAG1

The siRNA target sites within the human Ape1/Ref-1 and JAG1 genes were chosen by using Ambion's siRNA Target Finder program (Austin, TX). SiRNA target sequences are as follows: Ape1/Ref-1 siRNA (533 bp from the ATG), 5'-AAGTCTGGTACGACTGGAGTA-3' and JAG1 siRNA (2,623 bp from the ATG) 5'-AAATGGGATGATGACTGTAAT-3'. The control siRNA (AM4611) was purchased from Ambion. The control siRNA served as a negative control. The siRNAs were prepared by using a transcription-based method with a Silencer siRNA construction kit (Ambion). The cells were transfected with the siRNA duplexes using Lipofectamine RNAiMAX (Invitrogen).

3. Immunoblot analysis

Whole-cell lysates were prepared in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 % NP-40, 5 mM EDTA, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and 1×

protease inhibitor cocktail (Roche Diagnostics Corporation,Indianapolis, IN, USA)]. Protein concentrations from each sample were determined using the Bradford protein assay. Equal amounts of protein were loaded onto a 10% SDS-polyacrylamide gel, separated by electrophoresis, and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA). Following transfer, the membrane was blocked with 5% skim milk for 1 h and then incubated for 16 h with an anti-Ape/Ref-1 (sc-13104, Santa Cruz Biotechnology), anti- β-actin (sc-47778; Santa Cruz Biotechnology), anti- JAG1 antibody (sc-8303, Santa Cruz Biotechnology). The membranes were then washed, incubated with the appropriate secondary antibodies (1:4,000) in a blocking buffer for 2 hours, and washed again. The blotted proteins were detected using an enhanced chemiluminescence detection system (iNtRON Biotech, Seoul, Korea).

4. RT-PCR analysis

Total RNA was extracted from cells using the RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer′s protocol. The following primers were used: APE1 forward (5'-ATGCCGAAGCGTGGGAAAAA-3') and APE1 reverse (5'-TCACAGTGCTAGGTATAGGGTG ATAGG-3') (designed to amplify a 957-bp region); Redox APE1 forward (5'-ATGCCGAAGCGTG GGAAAAAG-3') and Redox APE1 reverse (5'-TCATGAACATTTGGTCTCTTG-3') (designed to amplify a 300-bp region); Repair APE1 forward (5'-GAGAACAAACTACCAGCTGAA-3') and Repair APE1 reverse (5'-TCACAGTGCTAGGTATAGGGT-3') (designed to amplify a 656-bp region); and GAPDH forward (5'-TGACCACAGTCCATGCCATC-3') and GAPDH reverse (5'-TTACTCCTTGGAGGCCATGT-3') (designed to amplify a 492-bp region). To avoid overamplification, aliquots of each reaction mixture were retrieved after 25, 30, and 35 cycles for analysis by agarose gel electrophoresis and ethidium bromide staining.

*5. MTT (*3-94,5-dimethylthiazole-2-yl)-2,5-diphrnyl tetrazolium bromide) *assay*

Cells were cultured in serum free medium for 12 h. The cells (30-50% confluence) were then treated with siRNA transfection in serum-free medium for 24hr, 48hr, 72hr times. Ape1/Ref-1 siRNA, or JAG1 siRNA, and nonspecific control siRNA. Cell viability was determined by MTT assay. After treatment, 20㎕ of MTT (1mg/ml) in PBS was incubated with cells in a 48-well plate for 4 h at 37℃. Subsequently, the medium containing MTT was removed, and 200㎕ of DMSO (dimethyl sulfoxide) added. Cells were incubated for a further 10 min at 37°C with gentle shaking. The absorbance was read on an ELISA plate reader using a 540nm filter.

6. Flow cytometric analysis

Control siRNA, JAG1 siRNA or APE1 siRNA-transfected M059K cells were trypsinized, collected and washed twice with PBS. Cell pellets were fixed in 70% ethanol and stored at 4°C. For the analysis, cells were collected by centrifugation and the pellets were resuspended in 0.05 mg/ml of propidium iodide containing 0.6% Nonidet P-40. RNase (0.02 mg/ml) was added and the suspension was incubated in the dark at room temperature for 30 min. The cell suspension was then filtered and analyzed for DNA content on a FACScan (BDIS, San Jose, CA) flow cytometer. The percent of cells in different phases of the cell-cycle was analyzed with CELLQUest software (BDIS) using a Power Macintosh 7500/100 computer (Apple Computer, Cupertino, CA).

7. In vitro Migration assay using transwell

In vitro migration experiments were performed using transwell (Costar), which consist of a 24-well companion plate with cell culture inserts containing 8 mm pore size filters. Briefly, transfected M059K cells (2×10^3) with serum-free medium were added to each insert (upper chamber), and the chemoattractant (10% FCS) was placed in each well of a 24-well companion plate (lower chamber). After 24h incubation at 37°C in a 5% CO_2 incubator, the upper surface of the filter was wiped with a

cotton-tipped applicator to remove nonmigratory cells. Cells that had migrated through the filter pores and attached on the under surface of the filter were fixed and stained. The cells from 5 random microscopic fields were counted. All experiments were run in triplicate.

8.Statistical analysis.

All values are expressed as means ± s.d. Where indicated, I performed analyses of significance by the two-tailed Student's *t*-test. I considered $P<0.01$ (**) as highly significant.

III. RESULTS

1. Genes induced with APE by DNA microarray screening.

Lots of cancers were related with APE/Ref-1. I think APE/Ref-1 has something roles as controlling genes induced in cancer progression. To identify the genes regulated with APE, first, I cloned stably APE-expressed cell line and performed DNA microarray analysis (Fig.1). Significantly expressed-genes selected by fold-change were visualized by hierarchical cluster analysis. JAG1, Notch1 and Hes1 in APE-expressed cell were highly up-regulated in compared with control.

2. APE/Ref-1 is an upstream of JAG1

From DNA microarray results, JAG primer were designated and PCR were performed. In result, JAG1 mRNA and protein expression level were dramatically induced in APE-overexpressed and redox-only expressed cell line (Fig. 2). However, in pcDNA3.1 (Mock) and repair-only expressed cell line, JAG1 mRNA and protein nearly were expressed. To test whether JAG1 expression could be attributed to APE1 in cells, I examined endogenous levels of Ape1 and JAG1 in cancer cell lines. I observed that, although cell express APE1 and JAG1, Notch1 activation is a proportional correlation between APE1 and JAG1. In APE1-overexpressed GM00637 cells, JAG1 was induced and intracellular domain of Notch1 was activated and translocated in nucleus. Whereas, JAG1 siRNA treatment in GM00637/APE did not express $Notch1^{IC}$ as well as translocation (Fig.3). When APE1-overexpressed cell treat GSI (indicated concentration 5, 10, 20 and 50uM), $Notch1^{IC}$ expression was decreased (Fig. 6).

3. Hes1, the effect of Notch1 induces cell migration.

Hes1 is an essential process in physiological conditions such as wound healing, tissue regeneration, and in pathological conditions such as tumor invasion. To investigate whether APE1 are contribute to

migration properties, I performed in vitro migration assay in GM00637/APE cell lines. APE1 induced Hes1 expression in GM00637/APE cell. In addition, when JAG1 siRNA in GM00637/APE was treated in cells, Hes1 expression was reduced (Figure.4, 6). Expression APE1 in GM00637 cell significantly enhanced the migration activity, whereas the migration of APE siRNA-treated GM00637/APE cell was reduced. Furthermore, inhibited expression of JAG1 caused reduction in migration activity compared with the migration activity caused by GM00637/APE cells (Figure.5).

And Also, the treatment of GSI in GM00637/APE reduced the migration ability. I observed that GSI clearly delayed the migration activity of the HES1 expression GM00637/APE cells (Fig.6).

4. Expression of APE1, JAG1, Notch^{IC} and Notch3^{IC} in glioma cell lines.

To test whether APE expression could be correlated with APE1 in glioma cells, I screened the four types of glioma cells. All of glioma cell lines, except U87, expressed JAG1 with APE (Fig. 7). Specifically, M059K highly expressed APE and JAG1. It seems that a proportional correlation between APE1, JAG1, Notch1 and Notch3 levels in glioma cells. To study the role of APE in glioma cell, I choose M059K cell and performed siRNA treatment. APE1 siRNA-treated M059K was suppressed JAG1 expression as compared with control. In addition, APE1 siRNA- or JAG1 siRNA-treated cell was reduced Notch1^{IC} expression (Fig. 8).

5. Down-regulation of APE1 reduces cellular proliferative ability in M059K.

To investigate whether APE1 and JAG1 affects cellular proliferation in M059K cells, MTT assay was performed. The proliferation rate of APE1 and JAG1 expressing M059K was significantly increased as compared with that of control cells. Importantly, the proliferation rate of M059K cells using APE1 or JAG1 siRNA was significantly reduced as compared with that of the control siRNA-treated cell (Fig.9). These findings strongly indicate that APE1 and JAG1 play an essential role in mediating the proliferative activity of M059K cell.

6. Down-regulation of APE1 reduces cellular migration ability in M059K.

To investigate whether APE1 are contribute to migration properties, I performed in vitro migration assay in M059K cell lines. The migration ability of APE or JAG1 siRNA-treated cell was significantly reduced as compared with control (Fig. 10).

7. APE1 and JAG1 siRNA induce S phase arrest in M059K cells.

To investigate the effects of APE1 and JAG1 on cell growth in more detail, I analyzed the effects of APE1 and JAG1 siRNA on the cell cycle distribution of M059K cells. The cell cycle distribution was monitored by flow cytometry (FACS) analysis after propidium iodide staining of the cellular DNA. As seen in Figure 10, in comparison with control siRNA-transfected cells, S phase arrest was increased in APE1 or JAG1 siRNA-treated cell lines (Fig. 11).

IV. DICUSSION

APE1 is important in mediating DNA binding of the AP-1 protein complex (19-22). This occurs via a posttranslational mechanism in which conserved cysteine residues in the DNA-binding domains of Fos and Jun proteins are reduced, allowing DNA binding to occur. In addition, APE1 phosphorylation was observed in vivo, but the acceptor residues have not been identified (23, 24). Upregulation of APE1 significantly potentiates the hypoxia-induced expression of a reporter construct containing the HIF-1-binding site (25), and APE1 is thought to be critical in the linking of two coactivator proteins, CBP/p300 and SRC-1, to HIF-1α (26, 27). Furthermore, APE1 is found to be a component of protein complexes that binds to negative calcium response element (nCaRE) (28), Ku70(Ku86) (29), and heterogeneous nuclear ribonucleoprotein L (30) in the promoter of the parathyroid hormone (PTH) gene, the renin gene, and the APE1 gene itself, where it may downregulate the expression of these genes (31, 32). Recent work suggests that APE1 is acetylated by CBP/p300 both in vivo and in vitro, and acetylation stimulates binding to nCaRE in the PTH promoter, leading to downregulation of the PTH gene (33). In addition, the presence of Ape1/Ref-1 in the hypoxia-inducible transcriptional complex is required for the apparent high-affinity association between HIF-1 and its DNA recognition sequence (34). Moreover, APE1 stably interacts with Y-box-binding protein (YB-1) and enhance its binding to the Y-box element, leading to the activation of the multidrug resistance gene *MDR1* (35). Therefore, it appears that APE1 stimulates the transcriptional activation by redox-dependent and redox-independent mechanisms. APE1 are mutated in a large proportion of human cancers, and APE pathways are activated by a variety of other mechanisms in many other cancers. Rational therapies that target APE1 downstream signaling molecules essential for malignant cancer cell behavior.. Because tumorigenesis contributes to most cancer deaths, and because therapies that target Ras and its downstream signaling pathways are under active development as anticancer agents, an understanding of the biological role of APE1 in the tumor

progression is clinically important. The mechanisms whereby APE1 oncogenes maintain the transformed characteristics of human cancer cells are poorly understood and may differ from those required for tumor initiation. APE1 is involved in the reduction of the Cys-62 residues of p50, which is essential for DNA-binding activity of NF-κB, suggesting APE1 may act as a redox-sensitive regulator of NF-κB (36-39). Previous in vitro studies using recombinant human Ape1/Ref-1 proteins suggest that cysteine 65 of human Ape1/Ref-1 is the redox-active site of Ape1/Ref-1 (40). However, others found that this cysteine residue is not involved in redox regulation (41, 42). More recently, Ando et al. (43) suggest that Ape1/Ref-1 acts as a "redox chaperone" that facilitates the reduction of redox-sensitive transcription factors by other reducing molecules such as glutathione and thioredoxin. These authors also demonstrated that redox chaperone activity of Ape1/Ref-1 is critical to NF-κB-mediated gene expression in human cells. In the present study, I demonstrated that the expression of APE1 in GM00637 cell was correlated to JAG1, Notch1 signaling (Fig.3). Also, I showed that APE1 increased in human glioma cells. (Fig.7, 8). I studied the correlation with JAG and APE in APE siRNA and JAG1 siRNA- transfected M059K cells. JAG1 expression was inhibited by APE siRNA in M059K cells (Fig.8). These findings showed there are correlations among them. The novel finding in the present study is that APE1 and JAG1 siRNA cause growth inhibition and S phase cell cycle arrest. JAG1 has been found to be up-regulated in prostate (43) and some other cancers such as glioma and breast cancer (44, 45). In the current study, I found that the siRNA of APE1 and JAG1 exerts a much stronger growth inhibitory effect on M059K cells. It has recently been shown that Jagged-1 is processed in a fashion similar to Notch-1, ultimately resulting in the release of a nuclear-targeted intracellular domain (46). Recently, the Jagged-1 intracellular domain has been shown to up-regulate activator protein 1 (AP-1) activity, a signaling pathway known to be important in many cancers. Whereas JAG1 plays a role in stimulation of Notch-1 in glioma cells, its significance in glioma cell biology may go beyond its effect on Notch-1 activation. I next investigated whether APE1 and JAG1 expression contributed to increase of cell proliferation , migration in glioma cells

(Fig. 9,10). I have shown that the si-RNA transfection of APE1 and JAG1 expressing cells led to decrease of the proliferation, migration and increase of the growth arrest, compared with those of the M059K cells (Fig.9, 10, 11). These results show that APE1 is strongly involved in increase of cellular proliferation, migration, growth arrest.

Therefore, my results show, for the first time, that APE regulates JAG1 expression to induce cell growth and migration in glioma cells. I suggest that APE regulates JAG1/Notch1 signaling to severe cancer progression occurring metastasis and tumor growth in the brain. To study the downregulation of APE will help to new cancer therapeutic approach.

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Figure legends

Fig. 1. DNA microarray from APE-transfected fibroblast cell lines.

High-quality data were analyzed using the Statistical Analysis of Microarrays software package Arrayassist® (Stratagene, La Jolla, CA). After selecting high-quality data, array elements that were 2-fold above or below the median on at least two microarrays were included (728 cDNA elements). These elements are displayed in hierarchical cluster format where rows represent genes and columns represent experimental samples. Colored pixels capture the magnitude of the response for any gene. Shades of red and green represent fold above and fold below the median, respectively. Black pixels reflect the median and gray pixels represent missing data. GM00637/pcDNA3.1 and GM00637/APE cells cells were analyzed in three of individual samples and each cell line in triplicate, and the sample dendrogram was generated by hierarchically clustering the arrays using the elements shown.

Fig. 2. The function of APE redox domain for JAG1 expression.

The structure of APE nuclease 1 (NCBI#, NP_001632.2) (a). APE primesr were designed and cloned for three of parts; redox (1~100bp, 11kDa), repair (101~318bp, 23.9kDa) and full-size one (1~318bp, 34.98kDa). (b) Total RNA was isolated GM00637(Mock), GM00637/APE. RT-PCR using the Redox APE1, Repair APE1, JAG1, β-actin primer. β-actin was used as the loading control. Amplified DNA products were separated on a 1.3% standard agarose gel and stained with ethidiumbromide. (c) Protein extracts prepared from GM00637cells stably expressing APE1 or pcDNA3 vector alone and the Redox APE1, Repair APE1, JAG1 and β-actin were analyzed by western blotting. . For checking APE redox and repair, 10ug of protein was loaded in 10% acrylamide gel. For checking JAG1 expression, 40ug of protein was loaded in 8% acrylamide gel. β-actin was used as the loading control.

Fig. 3. Immunoblot and Confocal immunofluorescence of JAG1/Notch1^{IC}.

In APE-overexpressed (a) or JAG1 siRNA-treated GM00637/APE(c), JAG1 and intracellular domain of Notch1 (Notch1^{IC}) expression was detected by immunoblot. β-actin was used as the loading control. In APE-overexpressed or JAG1 siRNA-treated cell, JAG1 expression (red, detecting cell membrane) and Activation of Notch1^{IC} (green, detecting nucleus) were visualized (c, d) by immunofluorescence confocal microscopy. All of cell was stained using DAPI (blue, detecting nucleus).

Fig. 4. RT-PCR of Hes1 expression in APE-overexpressed or JAG1 siRNA-treated cell lines.

(a) Total RNA was isolated from GM00637(Mock), GM00637/APE cells and JAG1-siRNA-treated cell lines. PCR for Hes1 or JAG1 was perforemed. GAPDH was used as the loading control. Amplified DNA products were separated on a 1.3% standard agarose gel and stained with ethidiumbromide.

(b) To study whether Hes1 was expressed, Protein extracts were prepared from APE1-overexpressed or JAG1 siRNA-treated cell lines.

Fig. 5. The role of APE in cell migration.

Cell migration abilities for APE-overexpressed or JAG1 siRNA-treated cell lines were analyzed using uncoated transwell cell culture inserts (2×10^4) with 8 μm pores. After 48 hours of incubation of cells, the cells that had invaded the lower surface of the membranes were fixed with methanol and stained with hematoxylin and eosin. The number of invading cells was determined by counting the cells that invaded to the lower side of the filter using a light microscopy at x40. Ten random fields were counted for each filter, and each sample was assayed in triplicate.

Fig. 6. RT-PCR of HES1 expression in GSI-treated cell line

(a) Gamma secretase inhibitor (GSI) was treated by 5, 10, 20 and 50. Twenty-four hour later PCR was performed for Hes1(Notch1 target). (b) For cell migration, cells ($2X10^4$/24h) were cultured in transwell after GSI (50uM) treatment, the cells that had invaded the lower surface of the membranes were fixed with methanol and stained with hematoxylin and eosin. The number of invading cells was determined by counting the cells that invaded to the lower side of the filter using a light microscopy at x40. Ten random fields were counted for each filter, and each sample was assayed in triplicate.

Fig. 7. Immunoblot of APE, JAG1, Notch1^{IC} and Notch3^{IC} in various glioma cell lines.

Expression of APE1, JAG1 and Notch1^{IC} and Notch3^{IC} in various glioma cells by immunoblot analysis. All of cells expressed APE1 , JAG1 and Notch1^{IC} and Notch3^{IC} except U87. β-actin was used as the loading control.

Fig. 8. RT-PCR and Immunoblot of APE, JAG1, Notch1^{IC} in M059k cell lines.

The indicated M059k cells were transfected for 48hr with control siRNA or APE1 siRNA or siJAG1. (a) Total RNA was isolated M059k cells. APE1, JAG1, β-actin primer. β-actin was used as the loading control. (b) Whole-cell lysates were prepared and subjected western blotting with anti-APE1, anti-JAG1, anti-Notch1 or anti-β-actin antibodies. P: control, CS: control siRNA, AS: APE1 siRNA, JS: JAG1 siRNA.

Fig. 9. Cell Proliferation Assay of APE, JAG1 in M059k cell lines.

Cells were grown for 24h, 48h, 72h at 37 ℃ and then labeled by adding 10 uM bromodeoxyuridine (BrdU) to each well flat bottoms at a density of 1000 cells 48 well.

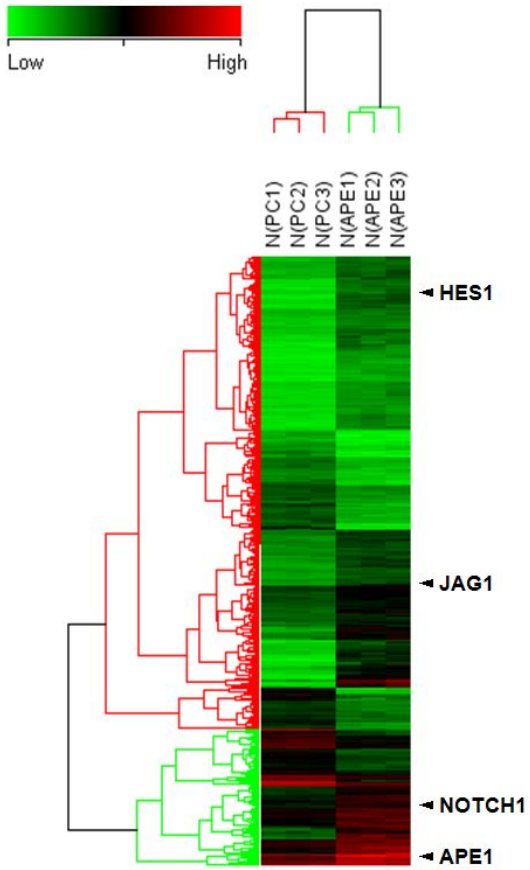
Fig. 10. *In vitro* cell migration in APE or JAG1 siRNA-treated M059K cell line.

Cell migration abilities of M059K, control siRNA, APE1 siRNA or JAG1 siRNA were analysed using uncoated transwell cell culture inserts (2×10^3) with 8 μm pores. After inhibition of cell

proliferation by treatment with 10μg/ml mitomycin-C or solvent were added to lower compartment. After 24 hours of incubation of cells, which had migrated through the pores. Cellular migration was observed with light microscope(×40). Three independent assays were performed in triplicate. Mean values ±s.d. are shown.

Fig. 11. Down-regulation of JAG1 induces S phase arrest in M059k cells.

Forty-eight hour after siRNA treatment, cells (1 X 10^5) were trypsinized and fixed in 70% ethanol. After washing, the cells were exposed to propidium iodide/RNase solution before performing flow cytometric analysis. Data shown are representative of 2 independent experiments. CS: control siRNA; JS: JAG1 siRNA; AS: APE siRNA; M1: G0-G1 phase; M2: S phase; M3: G2-M phase.



Group : Jagged 1/Notch1 signaling

SYMBOL	*Accession#*	*Fold (APE/PC)*
JAG1	NM_000214	3.57
HES1	NM_005524	3.41
Notch1	NM_017617	2.52

Fig. 1. DNA microarray data

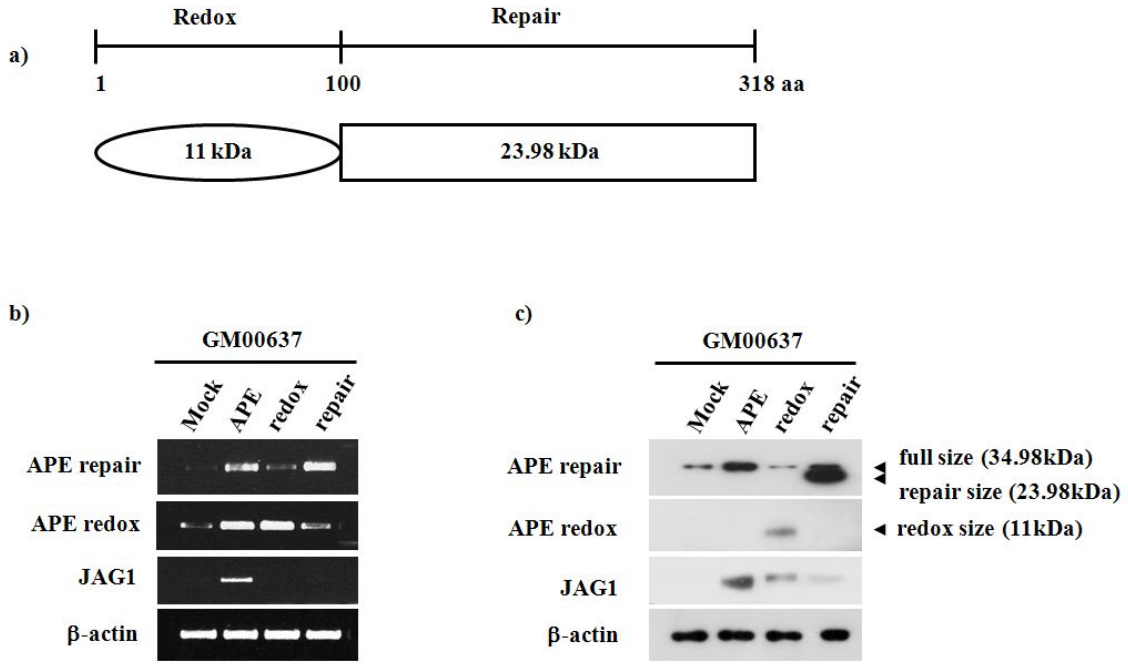


Fig. 2. The effect of APE for regulation of JAG1 expression

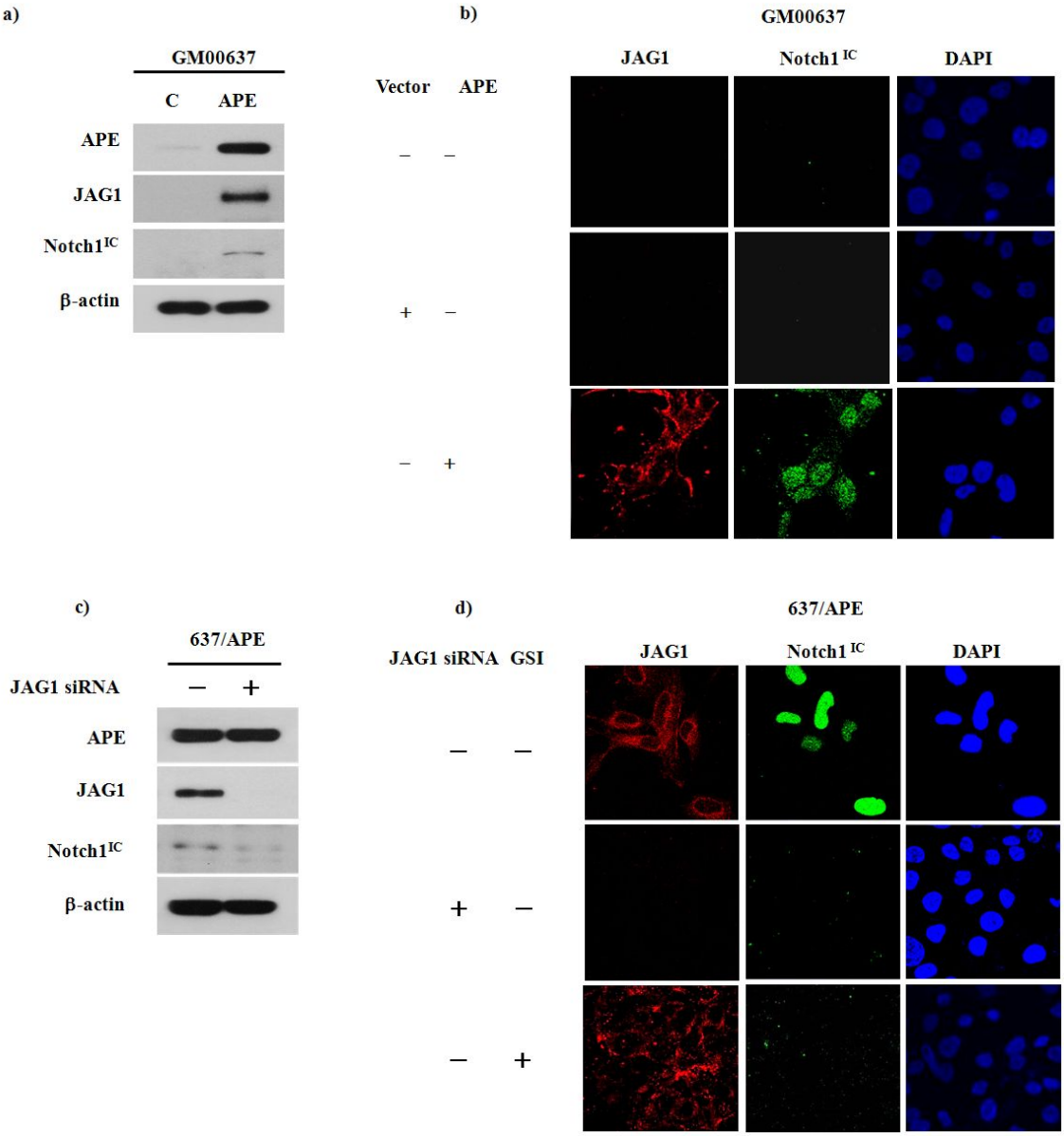


Fig. 3. Immunoblot and Confocal immunofluorescence of JAG1/Notch1^{IC}

Fig. 4. RT-PCR of Hes1 expression in APE or JAG1 siRNA-treated cell line

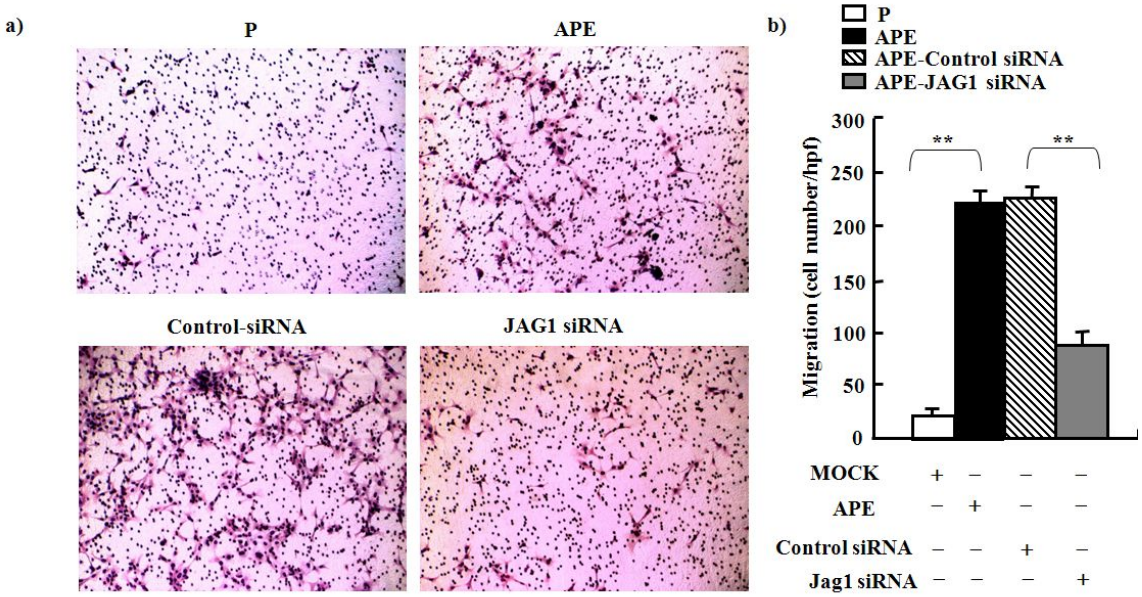


Fig. 5. In vitro cell migration ($2X10^4$/48h)

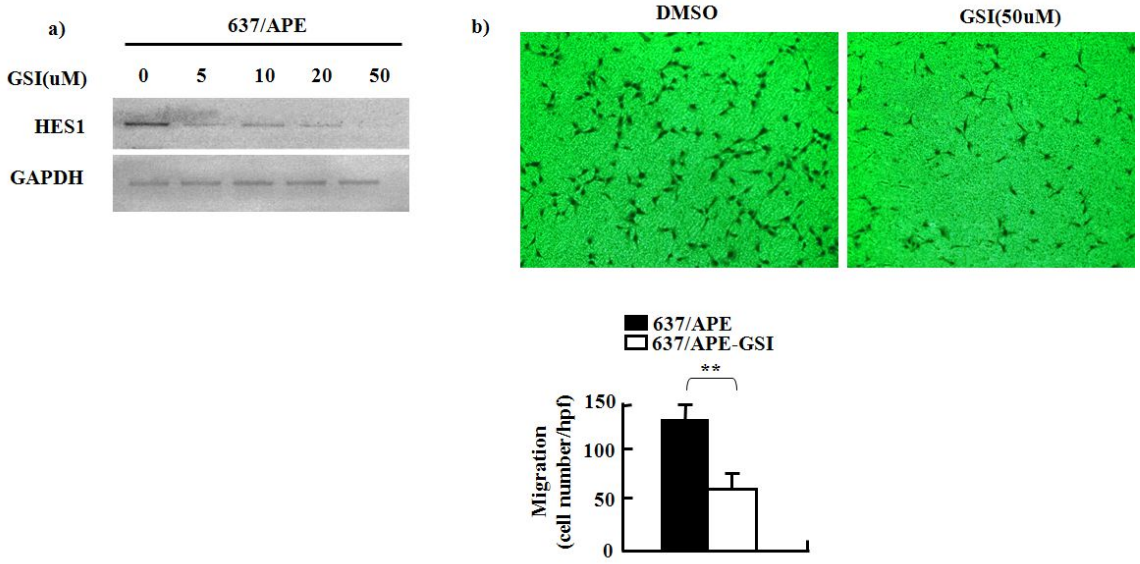


Fig. 6. RT-PCR of Hes1 expression in GSI-treated cell line

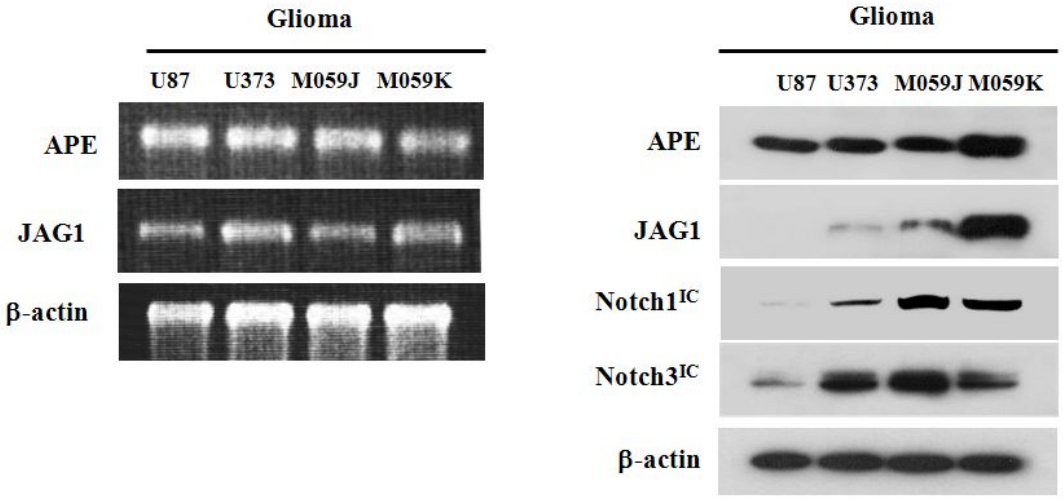


Fig. 7. Immunoblot of APE, JAG1, Notch1^{IC} and Notch3^{IC} in glioma cell lines.

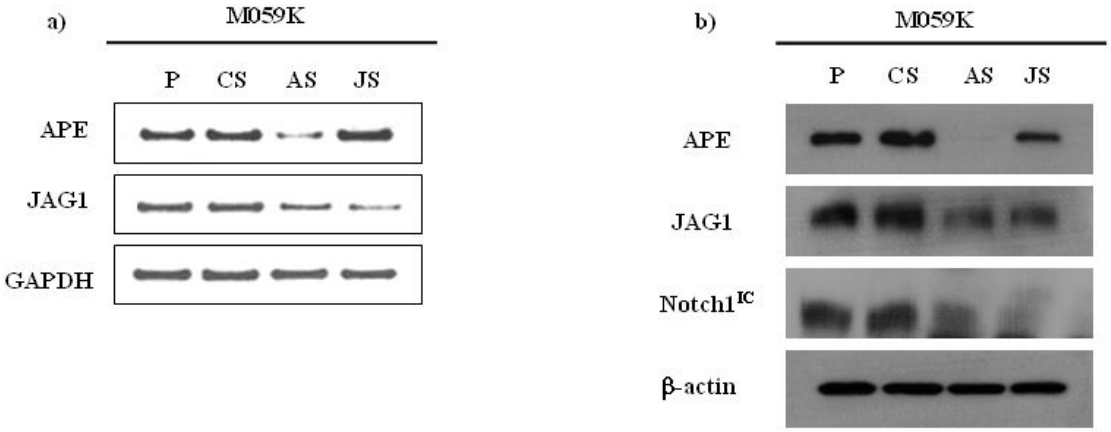


Fig. 8. RT-PCR and Immunoblot of APE, JAG1, Notch1^{IC} in M059k cell lines.

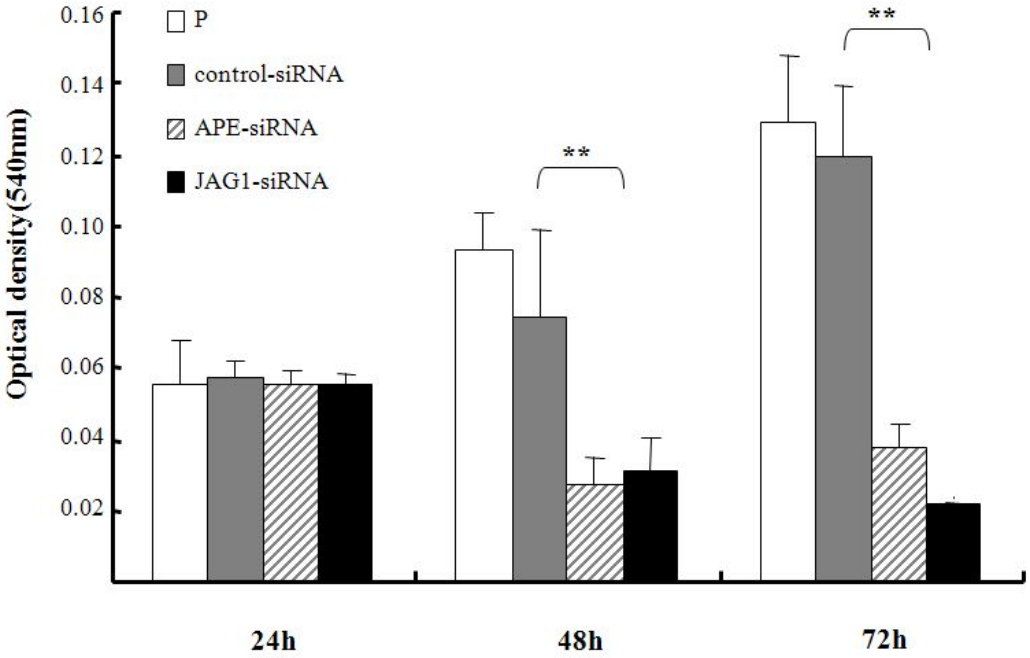


Fig. 9. Cell Proliferation Assay of APE or JAG1 siRNA-treated M059k cell lines.

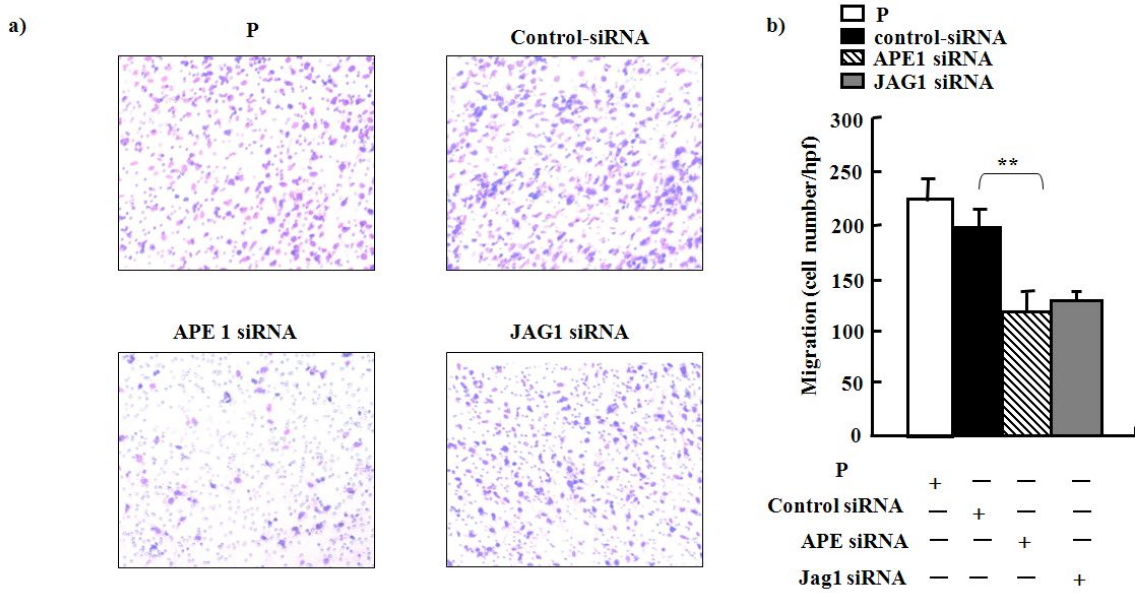
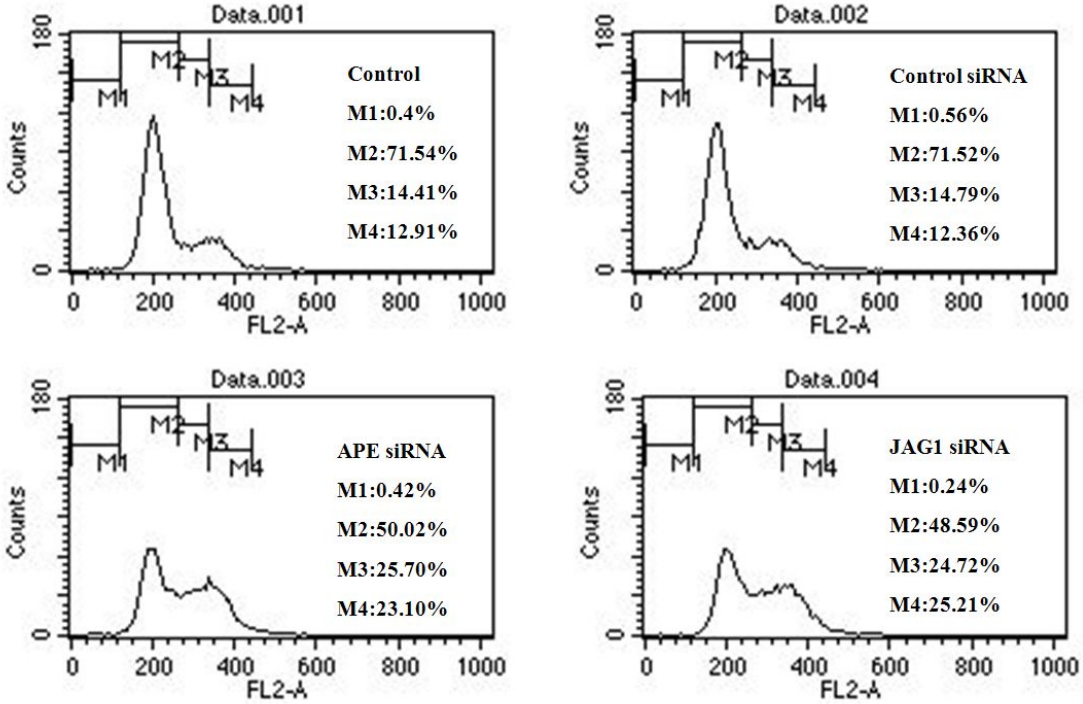


Fig. 10. ***In vitro*** **cell migration in APE or JAG1 siRNA-treated M059K cell line.**



Fig, 11. Down-regulation of JAG1 induces S phase arrest in M059k cells.

<국문초록>

Redox factor-1이 암 진행에 미치는 영향

정 성 미

(지도교수 : 장 인 엽)

조선대학교 대학원 생물신소재학과

APE/Ref-1는 redox signaling, DNA repair, 세포 자살 및 세포 증식에 포함되는 다기능 단백질이라고 알려져 있으며, APE1는 여러 암에서 높게 발현되어있다. 그러나 아직까지 암 진행과정에서 APE/Ref-1의 역할은 명확하지 않다. 본 저자는 암 진행과정에 있어서 APE/Ref-1의 기능에 대한 분자적 기작을 규명하고자 한다. APE/Ref-1에 의해 발현에 영향을 받는 유전자들을 찾기 위해 DNA microarray를 시행했으며 종양형성과 이동에 관여하는 JAG1이 관여되었음을 보였다. APE/Ref-1을 과발현시킨 섬유아세포에서 JAG1관련 유전자들이 발현되었고, APE발현을 억제하는 siRNA처리 후, JAG1관련유전자들의 발현과 활성이 급격히 감소함을 확인했다. 또한 신경교종세포의 성장과 이동에도 관여하는지 조사하기 위해 세포증식검사와 이동검사를 시행하였다. 그 결과 APE가 JAG1의 발현을 조절함으로써 세포증식과 이동에 관여하고 있음을 알게 되었고 세포주기에서 DNA합성단계인S-phase에 영향을 미친다는 것을 보였다. APE가 JAG1의 발현을 조절한다는 본 저자의 연구는 새로운 항암제 개발과 진단 및 치료에 대한 정보를 제공 함으로써 종양연구에 기여할 것이라 사료된다.

저작물 이용 허락서

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논문제목	한글 : Redox factor-1이 암진행에 미치는 영향 영문 : The effect of redox factor-1 on the cancer progrssion				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의 여부: 동의 (0) 반대 ( )

2009 년 10 월 19 일