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The identification of proliferative mechanism of redox factor

조선대학교 대학원

의학과

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이 논문을 의학석사학위신청 논문으로 제출함.

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The identification of proliferative mechanism of redox factor

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ABSTRACT

Human apurinic/apyrimidinic endonuclease (APE) is a multifunctional protein that is capable of repairing abasic sites and single-strand breaks in damaged DNA. In addition, it serves as a redox-modifying factor for a number of transcription factors. Identifying the transcriptional targets of APE is essential for understanding the mechanisms for how it affects various cellular outcomes. Expression array analysis was used to identify glial cell-derived neurotropic factor receptor α1 (GFRα1), which is an encoding receptor for the glial cell-derived neurotropic factor (GDNF) family, whose expression was induced by APE. It was also found that a target of GDNF/GFRα signaling, c-Src (Tyr418), was strongly phosphorylated by GNDF in

APE expressing cells. Moreover, it was found that GDNF could initiate cell proliferation in the APE expressing cells, which was measured by counting the number of cells. Importantly, the downregulation of APE by siRNA caused a marked reduction in the GFRα1 expression level, and reduced the ability of GDNF to phosphorylate c-Src (Tyr418) and stimulate cell proliferation. These results show an association between APE and GDNF/GFRα signaling, and suggest a potential molecular mechanism for the involvement of APE in cell survival and proliferation.

I. INTRODUCTION

APE 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 (11,12). This effect is mediated via the reduction of a conserved cysteine residue located at the DNA-binding domains of c-*fos* and c-*jun* (13-15). APE is also capable of modulating or activating other classes of transcription factors via a similar reducing action including NF-κB, p53, Egr-1, c-Myb, HLF, and Pax-8 (16-20). The ability of APE to activate the transcription factors involved in the cellular response to various stresses, suggests that APE may play an important role in various cellular processes.

It is not known why APE, which is vital to a critical DNA-repair process, can also affect the functioning of several apparently disparate transcriptional regulators. Whatever the significance of its different roles in cells may be, APE is essential for early development. This is because a deletion of the APE gene is lethal at a very early stage of embryogenesis (21). Moreover, APE has been implicated in the protection against cell death resulting from various toxic stimuli. The reduction of APE has been reported to sensitizing the cells against oxidative DNA damage (22,23). In contrast, APE overexpression provokes an increase in resistance to some alkylating agents and oxidative stress (24-26).

Although the DNA repair and transcription factor reducing properties of APE are well-known, other fundamental mechanisms by which it may regulate redox-sensitive

transcription, and influence cell function need to be elucidated. Identifying the transcription targets of APE is essential for understanding the pathways by which APE affects cellular outcomes. To date, the list of transcription targets of APE is not comprehensive. Expression array analysis was performed using Ad-APE (adenovirus encoding an *APE* gene) infected GM00637 human fibroblast cells was performed in an effort to identify the downstream target genes of APE particularly those that might be involved in APE-mediated cell survival and proliferation. This paper reports an APE target gene, glial cell-derived neurotropic factor receptor α1 (GFRα1), which were identified through this screening, contributes to the APE-mediated increase in glial cell-derived neurotropic factor (GDNF) responsiveness including c-Src activation and cell proliferation.

II. MATERIALS AND METHODS

1. Cell culture and Construction of adenoviral vector encoding hAPE cDNA.

The human fibroblast GM00637 cells (Coriell Institute for Medical Research) were maintained in Earle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS). The human pancreatic cancer cell line DU145, human colon cancer cell line DLD1 and human neuroblastoma cell line SKNSH (American Type Culture Collection) were maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS). The cells were maintained in 5%CO_2/95% air at 37 ℃ in a humidified incubator. Human *APE* cDNA, was amplified by RT-PCR using the *APE* oligo primer (5`-TCT AGA ATG CCG AAG CGT GGG AAA AAG G-3`, 5`-GGT ACC TCA CAG TGC TAG GTA TAG GGT G-3`) from human fibroblast GM00637 cells. The mutation of cysteine 65 in *APE* (APE^{C65}) was made by *in vitro* mutagenesis kit (Stratagene) using the primers (5`-AAA CCT GCC ACA CTC AAG ATC GCC TCT TGG AAT GTG GAT GGC TT-3`). The underlined letter indicates the nucleotide substitutions of the insert mutations (TGC→GCC). The nucleotide sequence of each construct was confirmed by cycle sequencing using an ABI PRISM 310 genetic analyzer (PerkinElmer Life Science). The cells were transfected with the Lipofectamine (Gibco BRL) according to the manufacturer's protocol.

The *APE* cDNA was cloned into a pcDNA3 mammalian expression vector (Invitrogen, Carlsbad, CA, USA) and a *pShuttle* vector (Invitrogen) after confirming the DNA sequence. The newly constructed plasmid *pShuttle-hAPE* was then doubly digested with *PI-SceI/I-CeuI*, and the purified product was ligated using Adeno-X DNA. The DNA was linearized with *PacI* and purified before Liopfectamine (Invitrogen) transfection of HEK293 cells. After transduction, HEK293 cells layers were overlaid with agarose and assessed for viral plaque formation at 10 days. For virus collection, the cells were lysed with three consecutive freeze-thaw cycles, and the virus was collected from the supernatant. The virus titer was approximately $1x10^7$ pfu/ml, which was determined using an end-point dilution assay. A vector carrying the β-galactosidase gene *LacZ* (Ad-LacZ) was used to monitor efficiency of transduction by the viral vectors and a nonspecific transgene expression controls. The transduction efficiency was tested by *in situ X-Gal staining*, and infection with 50-100 multiplicity of infection (MOI) of Ad-LacZ resulted in 90 – 100 % of cells testing positive in GM005637 human fibroblast cells.

2. Western Blotting

The cells were washed with PBS, and lysed at 0 °C for 30min in a M-PerR Mammalian Protein Extraction Reagent (Pierce, Rockford, IL, USA). The protein content was determined using a Bio-Rad dye-binding microassay (Bio-Rad, Hercules, CA, USA). 20 μg of protein per lane was electrophoresed on 10% SDS

polyacrylamide gels after boiling the protein in a Laemmli sample buffer for 5 min. The proteins were blotted onto Hybond-C membranes (Amersham Biosciences, Piscataway, NJ, USA), and the protein markers (Fermentas, Hanover, MD, USA) were used as the size standards. After electroblotting, the membranes were blocked with 1X Tris-buffered saline containing Tween-20 (TBS-T; 10 mM Tris-HCl, pH 7.4, 150mM NaCl, 0,1% Tween-20) and 5% milk, and incubated with the primary antibody diluted in a 1X TBS-T buffer for 2 h. The primary antibodies were diluted by 1/1000. The membranes were repeatedly washed and incubated with the appropriate secondary antibodies (1/4000) in a 1X TBS-T buffer for 1 h. The blotted protein was detected using an ECL kit (iNtRon Biotech, Korea). The following antibodies were used for immunohistochemistry and immunoblot analyses: rabbit polyclonal antibody GFRα1(H-70) and APE(C-20) (Santa Cruz Biotechnology, Inc) and phospho c-Src(tyr418) (Cell Signaling Technology, Beverly, MA, USA)

3. Immunofluorescence Microscopy.

The paraformaldehyde-fixed cells were incubated with anti-GFRα1 antibody (Santa Cruz Biotechnology). The cells were stained by incubation with fluorescein isothiocyanate (FITC)-conjugated anti-rabbit or anti-goat secondary antibodies (Vector, USA). The immunofluorescence images for the GFRα1 proteins were obtained using FV300 laser microscopy (Olympus, Japan) at an excitation wavelength appropriate for FITC (488nm).

4. Small interfering RNA (siRNA)-based Experiments

The siRNA target sites within the human *APE* and *GFRα1* gene were chosen using the Ambion's siRNA target finder program: APE siRNA (534bp from Atg) GUCUGGUACGACUGGAGUAtt-3' (sense) and 5'-UACUCCAGUCGUACCAGACtt-3' (anti-sense); GFRα1 siRNA(1228bp from Atg) 5'-UACACACCUCUGUAUUUCCtt-3' (sense) and 5'-GGAAAUACAGAGGUGUGUAtt-3' (antisense); LacZ siRNA, 5`-CGUACGCGGAAUACUUCGAtt-3` (sense), 5`-AAUC GAAGUAUUCCGCGUACGtt-3` (antisense) for the *LacZ* gene. These siRNAs were prepared using a transcription-based method with a Silencer siRNA construction kit (Ambion, Austin, TX, USA). The cells were transfected with the siRNA duplexes using Oligofectamine (Invitrogen).

5. WST-1 Proliferation Assays

GM00637 cells were infected with Ad-LacZ or Ad-APE. 24 h later, the cells were then incubated with or without GDNF (Sigma-Aldrich). To investigate the effect of APE and GRFα1 on the cell proliferation in response of GDNF, Ad-LacZ or Ad-APE infected cells were transfected with control siRNA, APE siRNA or GFRα1 siRNA using the Oligofectamine transfection reagent (Invitrogen). 24 h after transfection, the cells were then incubated with or without GDNF for up to 72 hours. Every 24 h after GDNF treatment, the WST-1 tetrazolium salt (Roche

Applied Science) was added to the culture for 2 h to monitor the level of cell proliferation according to the manufacturer's instructions. After the incubation period the production of formazan dye was quantified using a spectrophotometer (450 nm).

6. Statistical Analysis

All experiments were repeated at least three times to ensure reproducibility. Data in all experiments are represented as mean ± S.E. Statistical comparisons were carried out using two-tailed Student's *t* test. p values <0.05 were considered to be statistically significant.

III. RESULTS

1. APE induce expression of the GFRα1

The APE was expressed in the human fibroblast cells using the replication-deficient adenoviral vector harboring the human *APE* (Ad-APE) genes. The transduction efficiency of adenovirus vector in GM00637 human fibroblast cells was evaluated using a different adenovirus vector containing the β-galactosidase (Ad-LacZ) MOI and observing the X-gal staining of β-galactosidase 48 h after infection. It indicated that the cell infection rate increased with the increase of MOI of the viruses: MOI = 1, only a few cells expressed β-galactosidase; MOI = 50, approximate 90 % cells expressed β-galactosidase; MOI = 100, almost all cells expressed β-galactosidase (data not shown). The cells infected with 100 MOI of Ad-LacZ did not show the change in either the cell proliferation or morphology (data not shown). Based on the transduction efficiency of Ad-LacZ in GM00637 cells, cells infected with either Ad-APE or Ad-LacZ at the 50 MOI were used. To assess the adenovirus-mediated expression of APE, Western blot analysis was performed with extracts of cells 48 h after infection with an adenovirus vector, and a high-level of the APE protein was observed in the cells infected with the Ad-APE (Fig. 1A, *upper panel*).

In an attempt to identify the specific targets regulated by APE, cDNA microarray analysis was performed and the expression patterns in a human fibroblast cells GM00637 transfected separately with Ad-APE and Ad-LacZ were compared. The cDNA probes, prepared from the total RNA isolated from these cells, were labeled

and for hybridization with a human cDNA array containing the 8 K genes. (GenePlorer TwinChip 8 K, Digital Genomics, Seoul, Korea). Among the upregulated genes detected, we selected GFRα1 to investigate the biological function of APE for several reasons: first, GFR/GDNF signaling pathway promote the survival of various neurons, and have been expected as therapeutic agents for neurodegenerative disease; second, GFR/GDNF signaling pathway plays an important role of development, proliferation and differentiation in neuronal and non-neural tissues; third, APE is contributed to the cellular defense to particular types of genotoxic stress, and dysfunction of APE may be an underlying mechanism of neurodegenerative disease; fourth, APE is involved in the cell proliferation and development. To confirm this cDNA microarray result, semiquantitative RT-PCR analyses of the Ad-LacZ and Ad-APE infected fibroblast cells were performed. Semiquantitative RT-PCR analysis using the *GFRα1* primers showed that the expression level of the *GFRα1* genes was increased dramatically by infecting them with Ad-APE, but not with Ad-LacZ (data not shown). In order to determine if this increase in the *GFRα1* mRNA levels correspond to an increase in the GFRα1 protein level, western blots was carried out using an antibody against the GFRα1. SDS-polyacrylamide-gel electrophoresis (SDS-PAGE) was used to separate the whole-cell extracts of the protein from the APE-transfected cells, as well as to separate the protein from the LacZ-transfected cells. Western blot analysis with the GFRα1 antibody showed that the GFRα1 protein levels were higher in the APE-transfected cells than the LacZ-transfected cells. GFRα1

induction was observed as early as 24 h after the Ad-APE infection (Fig. 1A). Immunofluorescence staining using the same antibody also confirmed the expression of endogenous GFRα1 in Ad-APE-infected GM00637 cells but not in Ad-LacZ-infected cells (Fig. 1B).

To investigate whether APE can also induces GFRα1 expression in other human cells, Ad-APE or Ad-LacZ was infected into human pancreas cancer cell lines DU145, human colon cancer cell line DLD1 and human neuroblastoma cell line SKNSH. Forty-eight hours after infection, cells were harvested and GFRα1 expression levels were determined. Western blot analysis revealed that the GFRα1 expression levels in the three individual Ad-APE infected cells were significantly higher than that of the Ad-LacZ infected cells (Fig. 1C).

To investigate whether the endogenous APE protein affects the GFRα1 expression, GM00637 cells were treated with H_2O_2, which is known to be APE inducing agent. The GM00637 cells were treated with 100 μM H_2O_2 for 0, 12, 24 and 48 h, and the levels of the GFRα1 and APE expression were measured. As shown in Fig. 1D, treating the cells with H_2O_2 led to increase in the APE protein level within 24-48 h, and increases in the GFRα1 level were also observed 48 h after adding H_2O_2 to the medium. These results demonstrate that GFRα1 expression can be induced by endogenous APE.

2. c-Src becomes activated in APE expressing cells after GDNF stimulation

GFRα1 interacts with the GDNF family, resulting in the activation of the intracellular pathway, which contributes to cell proliferation, survival and differentiation (27-29). Therefore, this study investigated whether or not the GFRα1 induction mediated by APE could promote a functional interaction with GDNF. The receptor tyrosine kinase, Ret, is a major component in the signaling cascade activated by members of the GDNF family (30,31). However, Ret was not detected in the parental GM00637 cells, Ad-LacZ- and Ad-APE-infected cells (data not shown). Recent *in vitro* studies have shown that exogenously applied GDNF interacts with cells expressing GFRα1, leading to the activation of the Ret-dependent and Ret-independent signal pathways (32-34). Because Src-family kinase has been reported to be the direct downstream target of the GDNF/GFRα signal pathway in the Ret-deficient cell lines (32-34), this study examined whether or not GDNF induced Src activation by investigating the phosphorylation status of Src in the total lysates from the Ad-LacZ- and Ad-APE-infected cells using antibodies directed against the activated form of Src. As shown in Fig. 2, c- Src became phosphorylated on Tyr418 in the GNDF-stimulated, APE infected cells. The increase in c-Src phosphorylation was maintained for up to 3 h after the GDNF treatment. The levels of non-phosphorylated c-Src were unaffected by the GDNF treatment. Therefore, they were used as a control for equal loading. In contrast, the Ad-LacZ infected cells did not show c-Src phosphorylation at Tyr418 in response to GDNF (Fig. 2). Thus, APE-induced GFRα1 expression triggered the GDNF-mediated Src phosphorylation in the GM00637 cells.

3. GDNF treatment enhances cell proliferation in APE expressing GM00637 cells

It is known that the GDNF/GFRα system regulates cell survival and proliferation (27-29). Therefore, this study examined the effect of GDNF on the proliferation of Ad-APE and Ad-LacZ infected cells. Twenty-four hours after infecting the cells with either Ad-APE or Ad-LacZ, the cells were either left untreated or incubated with GDNF, and the number of cells was counted after a period of 1-3 days. As shown in Fig. 3, the Ad-APE infected cells treated with GDNF showed a more rapid increase in the number of cells on days 1, 2 and 3 than the Ad-LacZ infected cells treated with GDNF. These results suggest that the APE-mediated increase in GFRα1 expression results in the stimulation of cell proliferation in response to GDNF.

4. APE siRNA suppresses the GDNF responsiveness in Ad-APE infected GM00637 cells

In order to determine if APE indeed contributes to the enhancement of the GDNF responsiveness, small interfering RNA (siRNA) in the form of 21-base pair RNA duplexes, that target APE was used in an attempt to inhibit its expression level. The Ad-LacZ or Ad-APE infected cells were transfected with the mock, control siRNA oligonucleotide or the APE specific siRNA oligonucleotides. RT-PCR experiments were performed 48 h after treating the cells with either the APE siRNA or control

siRNA. The APE siRNA treatment resulted in a significant decrease in the APE mRNA level, compared with mock and control siRNA transfected cells (Fig. 4A). Western blot analysis revealed that the APE-specific siRNA oligonucleotide levels had decreased by more than 80 % in terms of their overall APE protein expression levels compared with the control siRNA-transfected cells (Fig. 4B). The GFRα1 levels after APE-siRNA transfection were next examined. The results showed that the Ad-APE-infected cells transfected with the APE-siRNA had significantly lower GFRα1 levels than the mock and control siRNA-transfected cells (Fig. 4A-B). The GNDF-induced c-Src activation and cell proliferation after APE siRNA transfection was next tested. As shown in Fig. 4C, the GDNF-induced c-Src (Tyr418) phosphorylation was suppressed in the Ad-APE-infected cells treated with the APE siRNA. The cell proliferation experiments confirmed that the transfection of APE siRNA strongly reduces the level of cell proliferation in response to GDNF compared with the control siRNA transfection (Fig. 4D).

IV. DISCUSSION

Ad-APE (adenovirus encoding a human APE)-infected GM00637 human fibroblast cells were used in this study to examine the effect of APE on gene expression. The results showed that APE mediates the increase in the *GFRα1* mRNA, GFRα1 promoter activity and GFRα1 protein levels, which is a key receptor for the glial cell-derived neurotropic factor (GDNF) family. It was further shown that c-Src, a downstream target of GFRα1, is functionally activated by GDNF in APE expressing cells, as determined by its phosphorylation. Moreover, it was found that GDNF could stimulate cell proliferation in the APE expressing cells, as measured by counting the number of cells. APE specific RNA experiments demonstrated that the downregulation of APE by siRNA caused a marked reduction in the GFRα1 expression level, as well as the diminished ability of GDNF to phosphorylate c-Src (Tyr418) and to stimulate cell proliferation. These findings suggest that GFRα1 is a direct target of APE.

The GDNF was originally characterized as a potent neurotropic factor specific for the survival and differentiation of the midbrain dopaminergic neurons (35). Subsequently, the biological effects of GDNF on the uterine branching in kidney morphogenesis, spermatogenesis, and survival as well as the differentiation of several other neuronal populations have considerably extended the range of activities of this polypeptide (36-38). Currently, four GFRα proteins, GFRα1, 2, 3, and 4 have been identified. GFRα1 mainly binds GNDF, and GFRα2, 3, and 4 bind

neurturin (NTN), artemin (ART), and persephin (PSP), respectively, which are the GDNF family of growth factors (39-43). The GDNF protein signals through a multi-component receptor complex, which consists of a glycosyl-phosphatidylinositol (GPI) binding subunit, which is known as the GDNF family receptor α (GFRα), and the transmembrane receptor tyrosine kinase (Ret) (27-29). This study demonstrated the functional involvement of APE in the GDNF/GFRα signal pathway. The induction of GFRα1 correlated with the initiation of signaling downstream of the GDNF in the APE expressing cells. Src was phosphorylated by GDNF in the APE-expressing cells. This agrees with a recent report showing that GDNF triggers Src-family kinase activation through GFRα1 independently of Ret (32-34). This suggests that APE can trigger the GDNF/GFRα signal pathway indicating that APE plays a role in cell survival and proliferation, as well as in normal development by modulating the GDNF/GFRα signal pathway.

GDNF-mediated activation of the GFRα/Ret system induces the subsequent signal transduction pathway and transactivation of its target genes, which leads to cell survival and proliferation (27-29). Most of the existing data on the biological effects of GDNF/GFRα were observed in the neuronal cells. Although the biological effects in non-neuronal cells are still unclear, several studies have indicated that GDNF/GFRα/Ret system might be involved in tumor cell proliferation, invasion and migration. For example, older mice overexpressing GDNF develop testicular carcinoma after one year of age as a result of an invasion of undifferentiating spermatogonia to the interstitium, suggesting that the

GDNF/Ret/GFRα signal pathway might be implicated in human germ cell carcinogenesis (44). In addition, the pancreatic cancer cell line contained both GFRα1 and Ret and GDNF increased the invasive capacity of human pancreatic cancer cell lines (45). Despite finding no GFRα1 expression in the normal bile duct, it was expressed clearly in a bile duct carcinoma, indicating that carcinogenesis leads to the aberrant expression of GFRα1 (46). Interestingly, a significant increase in APE expression has been demonstrated in malignant tissues, such as epithelial ovarian cancers, cervical cancer tissues and cell lines, prostate cell tumors, gliomas, rhabdomyosarcoma and germ cell tumors (47-52). A higher APE expression level was also reported to be associated with tumor progression (7). Therefore, the APE-mediated increase in the GDNF responsiveness, via GFRα1, might be an underlying mechanism of the migratory and invasive behavior of cancer cells.

During development, high level of APE expression is present in all somatic tissues (53). The presence of widespread and high level of APE expression during development is expected to play an important role in embryogenesis. *APE* null mice exhibits die during the embryonic stage, which results from a developmental defect (21). The phenotype of embryonic death observed in the *APE*-/- mice may be a consequence of defective DNA repair as well as inappropriate gene regulation whose expression is dependent on APE. This study demonstrated that a defect in APE expression by siRNA suppressed GFRα1 expression and the GDNF responsiveness. Mice lacking GDNF (54,55) and GFRα (56,57) all die soon after birth and share a similar phenotype of kidney agenesis and absence of enteric

neurons below the stomach, suggesting GNDF/GFRα signaling pathway plays an important role in morphogenesis during embryonic development. Although little is known about why *APE* null mice are embryonic lethal, one may speculate that arising from APE functional defect in APE-null embryos, a failure of GDNF/GFRα signal pathway needed to stimulate morphogenesis may contribute to embryonic death.

In conclusion, this study showed that the GDNF receptors, GFRα1, are induced by APE. It was also demonstrated that APE activates the GDNF responsiveness through GFRα1, resulting in c-Src phosphorylation and cell proliferation in the GM00637 human fibroblast cells. These results highlight the potential role of APE in normal development and cell proliferation mediated through GDNF/GFRα signaling.

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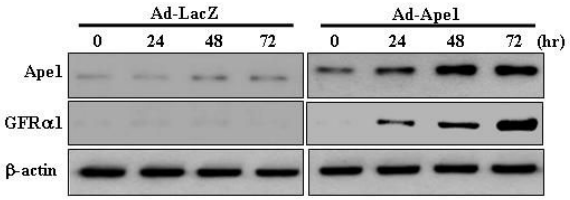
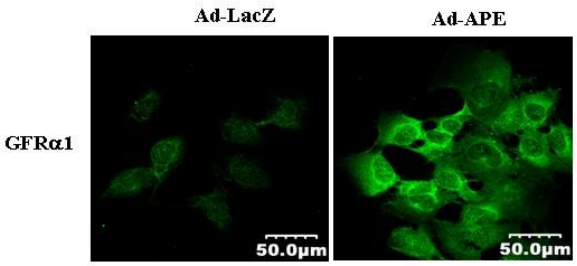
A


Ad-LacZ
Ad-Ape1
0 24 48 72 0 24 48 72 (hr)
Ape1
GFRα1
β-actin

B


Ad-LacZ
Ad-APE
GFRα1
50.0μm
50.0μm

C

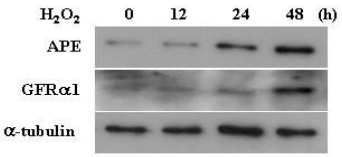

H2O2 0 12 24 48 (h)
APE
GFRα1
α-tubulin

Fig. 1. GFRα1 expression after expression of APE. (A) The GM00637 cells were infected with Ad-LacZ or Ad-APE, and the cells were then harvested at the indicated times after the infection. The cell extracts (20μg) were separated by electrophoresis on a 10% SDS/acrylamide gel and analyzed by Western blotting using the APE, GFRα1 antibody. α-tubulin was used as the loading control. (B) Immunofluorescence analysis of GFRα1 protein expression of the GM00637 cells 48 h after infecting them with Ad-LacZ or Ad-APE. The cells were stained with either the GFRα1 antibody. (C) DU145, DLD1 and SKNSH cells were transfected with Ad-LacZ or Ad-APE at a m.o.i. of 50, and the cells were harvested 48 h after the infection. Protein extracts prepared 48 h after the infection with Ad-LacZ or Ad-APE. Twenty μg of the total protein was loaded on SDS polyacrylamide gel for western blot analysis. The antibodies against APE and GFRα1 were used. The detection of α-tubulin was used as the loading control. (D) The HCT116 cells were treated with 100 μM H_2O_2 and lysed at the indicated times. The total protein was extracted and quantified as described in the "Experimental Procedures." Anti-GFRα1 and anti-APE antibodies were used to evaluate the GFRα1 and APE levels after the H_2O_2 treatment. α-tubulin was used as the loading control.

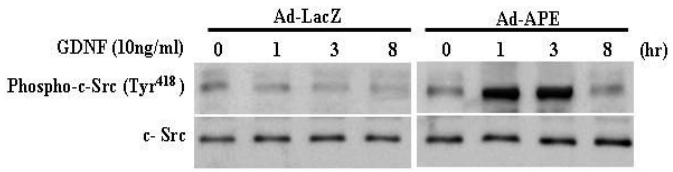


Fig. 2. GDNF-induced c-Src phosphorylation at Tyr418 in Ad-APE-infected cells. The GM00637 cells were infected with either Ad-LacZ or Ad-APE. 48 h after the infection, the cells were incubated with GDNF (10ng/ml) for the indicated time points. The total cell extracts were separated by 10% SDS-PAGE, electroblotted onto nitrocellulose membranes, and probed with anti-phospho c-Src (Tyr418) antibody. The bottom panels show the reprobing of the same filter with anti-c-Src antibody.

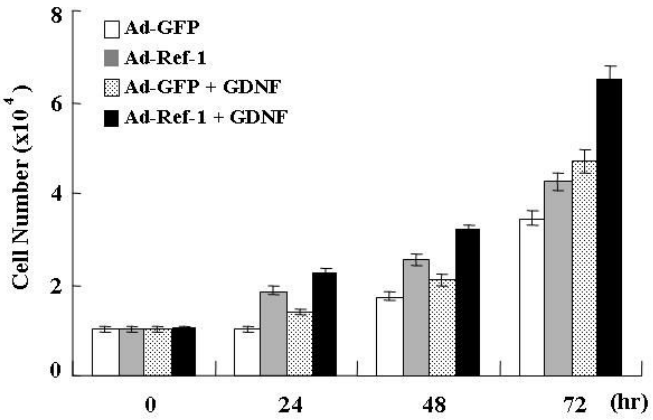
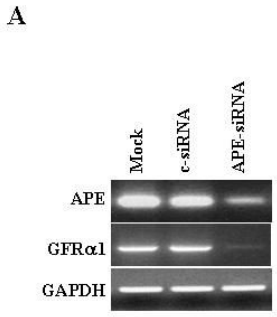
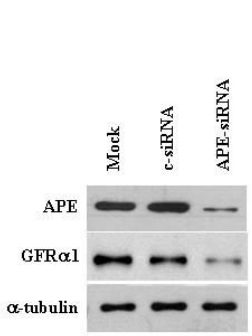
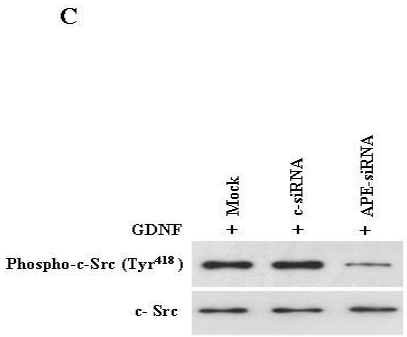


Fig. 3. GDNF treatment leads to cell proliferation in GM00637 cells in Ad-APE infected cells. The GM00637 cells were infected with Ad-LacZ or Ad-APE. 24 h after infection, the cells were then incubated with or without GDNF (10 ng/ml) for up to 72 hours. The number of cells was determined by counting the cells every 24 h after GDNF treatment. Each value is a mean ± SD from three separate experiments. The asterisk indicates significantly different from the Ad-LacZ + GDNF at $p < 0.01$.


A
Mock
c-siRNA
APE-siRNA
APE
GFRα1
GAPDH


B
Mock
c-siRNA
APE-siRNA
APE
GFRα1
α-tubulin


C
Mock
c-siRNA
APE-siRNA
GDNF
+
+
+
Phospho-c-Src (Tyr418)
c- Src

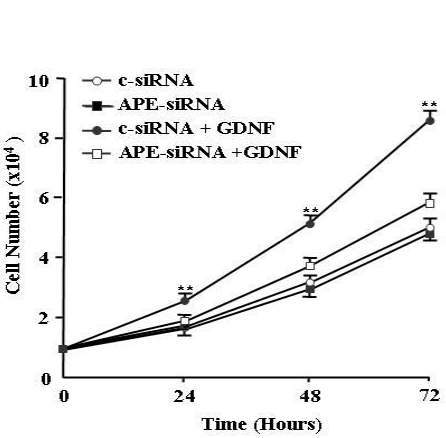

D
c-siRNA
APE-siRNA
c-siRNA + GDNF
APE-siRNA +GDNF
Cell Number (x10⁴)
Time (Hours)
0
2
4
6
8
10
0
24
48
72
**
**
**

Fig. 4.. Reduced APE associated with the suppressed GDNF responsiveness in Ad-APE infected GM00637 cells. (A) The Ad-LacZ or Ad-APE infected GM00637 cells were transfected with the mock, control siRNA or APE siRNA. 48 h after transfection, the total RNA was extracted from the cells and analyzed by semiquantative RT-PCR using the *APE*-or *GFRα1*-specific primers on 26 cycles. (B) The total protein was extracted from the cells and equal amounts (20 μg proteins) of the cell lysates were separated by 10% SAS-PAGE, and then transferred onto a nitrocellulose membrane. The membrane was immunoblotted with anti-APE, anti-GFRα1 or anti-α-tubulin antibodies. (C) The Ad-LacZ or Ad-APE infected GM00637 cells were transfected with the mock, control siRNA or APE siRNA and incubated with GDNF (10ng/ml) for 1 hr. The total cell extracts were separated by 10% SDS-PAGE, electroblotted onto nitrocellulose membranes, and probed with anti-phospho c-Src (Tyr418) antibodies. The bottom panels show the reprobing of the same filter with anti-c-Src antibody. (D) The Ad-LacZ or Ad-APE infected GM00637 cells were transfected with the mock, control siRNA or APE siRNA and incubated with GDNF (10 ng/ml) for up to 72 hours. The number of cells was determined by counting the cells every 24 h after GDNF treatment. Each value is a mean ± SD from three separate experiments. The asterisk indicates significantly different from the APE-siRNA + GDNF at $p < 0.01$.

<국문초록>

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의학과

산화환원인자인 APE/REf-1 은 일반적으로 유전자의 AP 부위를 정산화시키는 유전자손상 복구 단백질로 알려져있다. APE/REf-1 단백질은 다른 연구구룹에서 산화환원 조절인자로서의 기능이 있음이 밝혀져서 산화환원인자로 또한 불리운다. 산화환원인자인 APE/REf-1은 전사활성조절을 통하여 다양한 유전자 발현을 조절한다. 따라서 산화환원인자인 APE/REf-1는 다양한 유전자 산물들을 통하여 세포내에서 여러가지 생리학적인 역활을 할것으로 여겨진다.

APE/REf-1의 알려진 세포내 생리학적인 역활중 하나가 세포 분열을 조절한다고 알려져있다. 하지만 APE/REf-1 에의하여 조절되는 어떠한 단백질이 세포분열을 조절하는지는 알려져있지 않다. 따라서 본 연구 논문에서는 APE/REf-1에 의하여 세포분열 조절이 어떠한 기작을 통하여 이루어지는지를 연구 하였다. 연구

결과 APE/REf-1 는 세포내 GFR의 전사발현을통하여 세포분열을 조절함을 관찰하였다. 그결과 APE/REf-1 는 GFR의 downstream 신호 전달 경로로 알려진 c-Src의 인산화를 조절하여 세포분열으 조절함을 규명하였다. 본연구결과는 향후 APE/REf-1 와 관련된 암억제 방법을 개발하는데 중요한 단서를 제공 할 수 있을 것으로 판단된다.

저작물 이용 허락서

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논문제목	한글 : 산화환원 인자에 의한 세포 분열 조절 기작 연구 영문 The identification of proliferative mechanism of redox factor				

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 ( )

2010 년 8 월 일

저작자: 김 옥 현(서명 또는 인)

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