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APEX-1 Regulates Cell Proliferation through GDNF/ GFRα1 Signaling

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Human apurinic/apyrimidinic endonuclease (APEX-1) 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 APEX-1 is essential for understanding 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, the expression of which is induced by APEX-1. A target of GDNF/GFRα signaling, c-Src (Tyr418) was strongly phosphorylated by GNDF in the APEX-1 expressing cells. Moreover, GDNF initiated cell proliferation, measured by counting the number of cells, in the APEX-1 expressing cells. Importantly, the down-regulation of APEX-1 by siRNA caused a marked reduction in the GFRα1 expression level, and it reduced the ability of GDNF to phosphorylate c-Src (Tyr418) and stimulate cell proliferation. These results demonstrate an association between APEX-1 and GDNF/GFRα signaling and suggest a potential molecular mechanism for the involvement of APEX-1 in cell survival and proliferation.

Key words : Apurinic/apyrimidinic endonuclease (APEX-1), cell proliferation, glial cell-derived neurotropic factor (GDNF), glial cell-derived neurotropic factor receptor α1 (GFRα1), Src

Introduction

APEX-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 [22]. This effect is mediated via the reduction of a conserved cysteine residue located at the DNA-binding domains of c-*fos* and c-*jun* [5]. APEX-1 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 [5]. The ability of APEX-1 to activate the transcription factors involved in the cellular response to various stresses, suggests that APEX-1 may play an important role in various cellular processes.

It is not known why APEX-1, 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, APEX-1 is essential for early development. This is because a deletion of the APEX-1 gene is lethal at a very early stage of embryogenesis [23]. Moreover, APEX-1 has been implicated in the protection against cell death resulting from various toxic stimuli. The reduction of APEX-1 has been reported to sensitizing the cells against oxidative DNA damage [20]. In contrast, APEX-1 overexpression provokes an increase in resistance to some alkylating agents and oxidative stress [7].

Although the DNA repair and transcription factor reducing properties of APEX-1 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 APEX-1 is essential for understanding the pathways by which APEX-1 affects cellular outcomes. To date, the list of transcription targets of APEX-1 is not comprehensive. Expression array analysis was performed using Ad-APEX-1 (adenovirus encoding an *APEX-1* gene) infected GM00637 human fibroblast cells was performed in an effort to identify the downstream target genes of APEX-1 particularly those that might be involved in APEX-1-mediated cell survival and proliferation. This paper reports an APEX-1 target gene, glial

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cell-derived neurotropic factor receptor α1 (GFRα1), which were identified through this screening, contributes to the APEX-1-mediated increase in glial cell-derived neurotropic factor (GDNF) responsiveness including c-Src activation and cell proliferation.

Materials and Methods

Cell culture and construction of adenoviral vector encoding APEX-1 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 cells were maintained in 5% CO_2/95% air at 37°C in a humidified incubator. Human *APEX-1* cDNA, was amplified by RT-PCR using the *APEX-1* 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 cells were transfected with the Lipofectamine (Gibco BRL) according to the manufacturer's protocol.

The *APEX-1* 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-hAPEX-1* 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 1×10^7 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 GM00637 human fibroblast cells.

Western Blotting

The cells were washed with PBS, and lysed at 0℃ for 30 min 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, 150 mM NaCl, 0,1% Tween-20) and 5% milk, and incubated with the primary antibody diluted in a 1X TBS-T buffer for 2 hr. The primary antibodies were diluted by 1/1,000. The membranes were repeatedly washed and incubated with the appropriate secondary antibodies (1/4,000) in a 1X TBS-T buffer for 1 hr. 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 APEX-1 (C-20) (Santa Cruz Biotechnology, Inc) and phospho c-Src (tyr418) (Cell Signaling Technology, Beverly, MA, USA).

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 (488 nm).

Semiquantative reverse transcriptase polymerase chain reaction

The primers used for the PCR are as follows: *APEX-1* forward, 5′-ATG CCG AAG CGT GGG AAA AA-3′; *APEX-1* reverse, 5′-TCA CAG TGC TAG GTA TAG GGT GAT AGG-3; and GAPDH reverse 5′- CAA AGT TGT CAT GGA TGA CC-3′. The PCR products were resolved on 1% agarose gels, stained with ethidium bromide, and then photographed.

Small interfering RNA (siRNA)-based experiments

The siRNA target sites within the human *APEX-1* gene was chosen using the Ambion's siRNA target finder program: APEX-1 siRNA (534 bp from Atg) GUCUGGUACGACUGGAGUAtt-3′ (sense) and 5′-UACUCCAGUCGUACC-

AGACtt-3′ (anti-sense); LacZ siRNA, 5′-CGUACGCGGAA-UACUUCGAtt-3′ (sense), 5′-AAUC GAAGUAUUCCGC-GUACGtt-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).

Proliferation assays

GM00637 cells were infected with Ad-LacZ or Ad-APEX-1. 24 hr later, the cells were then incubated with or without GDNF (Sigma-Aldrich). To investigate the effect of APEX-1 and GRFα1 on the cell proliferation in response of GDNF, Ad-LacZ or Ad-APEX-1 infected cells were transfected with control siRNA or APEX-1 siRNA using the Oligofectamine transfection reagent (Invitrogen). 24 hr after transfection, the cells were then incubated with or without GDNF for up to 72 hr. Every 24 hr after GDNF treatment, the WST-1 tetrazolium salt (Roche Applied Science) was added to the culture for 2 hr 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).

Results

APEX-1 induce expression of the GFRα1

The APEX-1 was expressed in the human fibroblast cells using the replication-deficient adenoviral vector harboring the human *APEX-1* (Ad-APEX-1) 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 hr 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-APEX-1 or Ad-LacZ at the 50 MOI were used. To assess the adenovirus-mediated expression of APEX-1, Western blot analysis was performed with extracts of cells 48 hr after infection with an adenovirus vector, and a high-level of the APEX-1 protein was observed in the cells infected with the Ad-APEX-1 (Fig. 1A).

In an attempt to identify the specific targets regulated by APEX-1, cDNA microarray analysis was performed and the expression patterns in a human fibroblast cells GM00637 transfected separately with Ad-APEX-1 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 APEX-1 (Fig, 1B) 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, APEX-1 is contributed to the cellular defense to particular types of genotoxic stress, and dysfunction of APEX-1 may be an underlying mechanism of neurodegenerative disease; fourth, APEX-1 is involved in the cell proliferation and development. To confirm this cDNA microarray result, semi-quantitative RT-PCR analyses of the Ad-LacZ and Ad-APEX-1 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-APEX-1, 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 APEX-1-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 APEX-1-transfected cells than the LacZ-transfected cells. GFRα1 induction was observed as early as 24 hr after the Ad-APEX-1 infection (Fig. 1A). Immunofluorescence staining using the same antibody also confirmed the expression of endogenous GFRα1 in Ad-APEX-1-infected GM00637 cells but not in Ad-LacZ-infected cells (Fig. 1C).

To investigate whether the endogenous APEX-1 protein affects the GFRα1 expression, GM00637 cells were treated

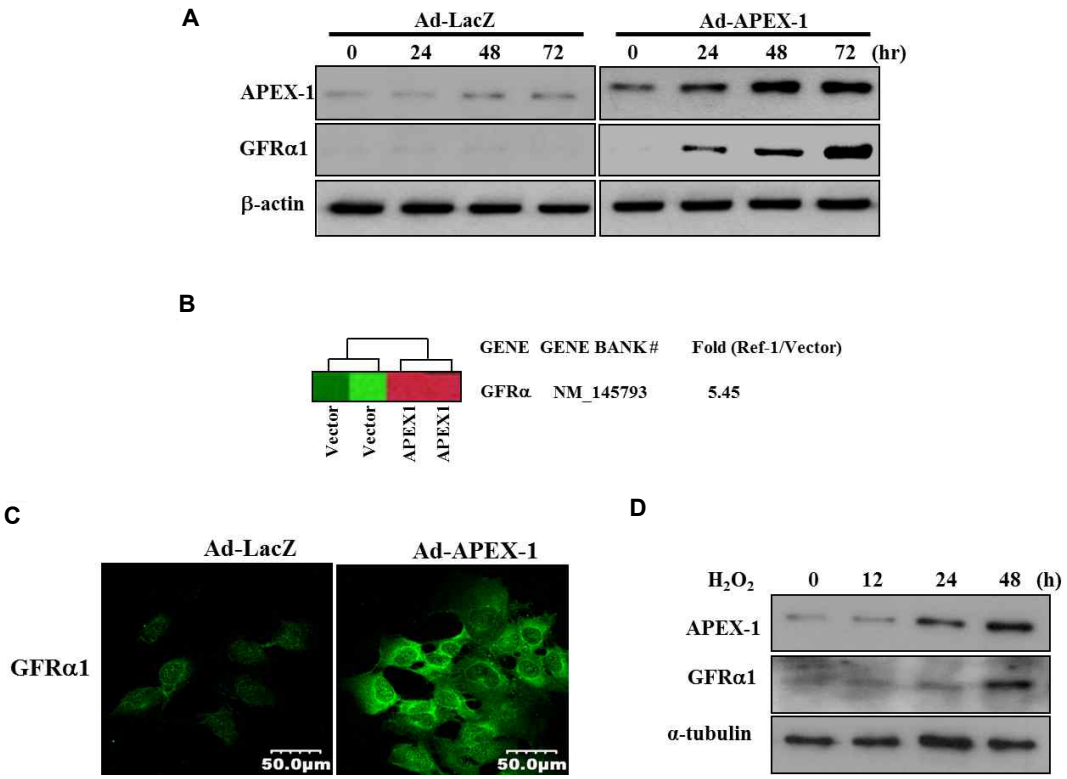

Fig. 1. GFRα1 expression after expression of APEX-1. (A) The GM00637 cells were infected with Ad-LacZ or Ad-APEX-1, and the cells were then harvested at the indicated times after the infection. The cell extracts (20 μg) were separated by electrophoresis on a 10% SDS/acrylamide gel and analyzed by Western blotting using the APEX-1, GFRα1 antibody. α-tubulin was used as the loading control. (B) Heat map shows that GFRα genes are induced by APEX1. (C) Immunofluorescence analysis of GFRα1 protein expression of the GM00637 cells 48 h after infecting them with Ad-LacZ or Ad-APEX-1. The cells were stained with either the GFRα1 antibody. (D) The GM00637 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-APEX-1 antibodies were used to evaluate the GFRα1 and APEX-1 levels after the H_2O_2 treatment. α-tubulin was used as the loading control.

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

c-Src becomes activated in APEX-1 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 [1]. Therefore, this study investigated whether or not the GFRα1 induction mediated by APEX-1 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 [9]. However, Ret was not detected in the parental GM00637 cells, Ad-LacZ- and Ad-APEX-1-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 [17]. 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 [17], this study examined whether GDNF induced Src activation by investigating the phosphorylation status of Src in the total lysates from the Ad-LacZ- and Ad-APEX-1-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, APEX-1 infected cells. The increase in c-Src

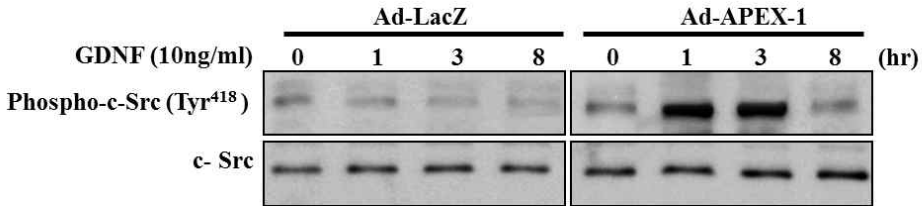

Fig. 2. GDNF-induced c-Src phosphorylation at Tyr418 in Ad-APEX-1-infected cells. The GM00637 cells were infected with either Ad-LacZ or Ad-APEX-1. 48 hr after the infection, the cells were incubated with GDNF (10 ng/ml) for the indicated time points. The total cell extracts were separated by 10% SDS-PAGE, 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.

phosphorylation was maintained for up to 3 hr after the GDNF treatment. The levels of nonphosphorylated 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, APEX-1-induced GFRα1 expression triggered the GDNF-mediated Src phosphorylation in the GM00637 cells.

GDNF treatment enhances cell proliferation in APEX-1 expressing GM00637 cells

It is known that the GDNF/GFRα system regulates cell survival and proliferation [1]. Therefore, this study examined the effect of GDNF on the proliferation of Ad-APEX-1 and Ad-LacZ infected cells. 24 hr after infecting the cells with either Ad-APEX-1 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-APEX-1 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 APEX-1-mediated increase in GFRα1 expression results in the stimulation of cell proliferation in response to GDNF.

APEX-1 siRNA suppresses the GDNF responsiveness in Ad-APEX-1 infected GM00637 cells

In order to determine if APEX-1 indeed contributes to the enhancement of the GDNF responsiveness, small interfering RNA (siRNA) in the form of 21-base pair RNA duplexes, that target APEX-1 was used in an attempt to inhibit its expression level. The Ad-LacZ or Ad-APEX-1 infected cells were transfected with the mock, control siRNA oligonucleotide or the APEX-1 specific siRNA oligonucleotides. RT-PCR experiments were performed 48 hr after treating the cells with either the APEX-1 siRNA or control siRNA.

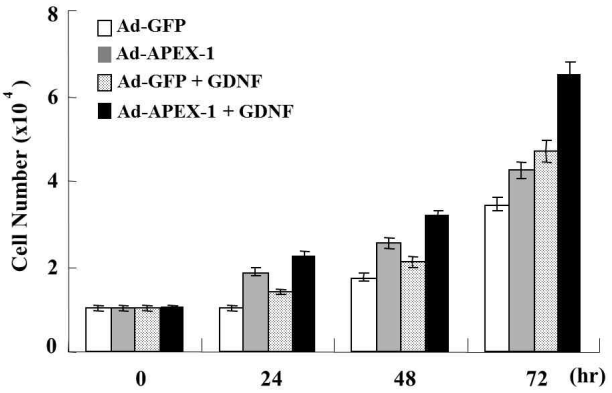

Fig. 3. APEX1 increases cellular proliferation in response to GDNF through GFRα1. GDNF treatment leads to cell proliferation in GM00637 cells in Ad-APEX-1 infected cells. The GM00637 cells were infected with Ad-LacZ or Ad-APEX-1. 24 hr after infection, the cells were then incubated with or without GDNF (10 ng/ml) for up to 72 hr. The number of cells was determined by counting the cells every 24 hr after GDNF treatment. Each value is a mean ± SD from three separate experiments. The asterisk indicates significantly different from the Ad-LacZ + GDNF at $p<0.01$.

The APEX-1 siRNA treatment resulted in a significant decrease in the APEX-1 mRNA level, compared with mock and control siRNA transfected cells (Fig. 4A). Western blot analysis revealed that the APEX-1-specific siRNA oligonucleotide levels had decreased by more than 90% in terms of their overall APEX-1 protein expression levels compared with the control siRNA-transfected cells (Fig. 4B). The GFRα 1 levels after APEX-1-siRNA transfection were next examined. The results showed that the Ad-APEX-1-infected cells transfected with the APEX-1-siRNA had significantly lower GFRα1 levels than the mock and control siRNA-transfected cells (Fig. 4A, 4B). The GNDF-induced c-Src activation and cell proliferation after APEX-1 siRNA transfection was next tested. As shown in Fig. 4C, the GDNF-induced c-Src (Tyr418) phosphorylation was suppressed in the Ad-

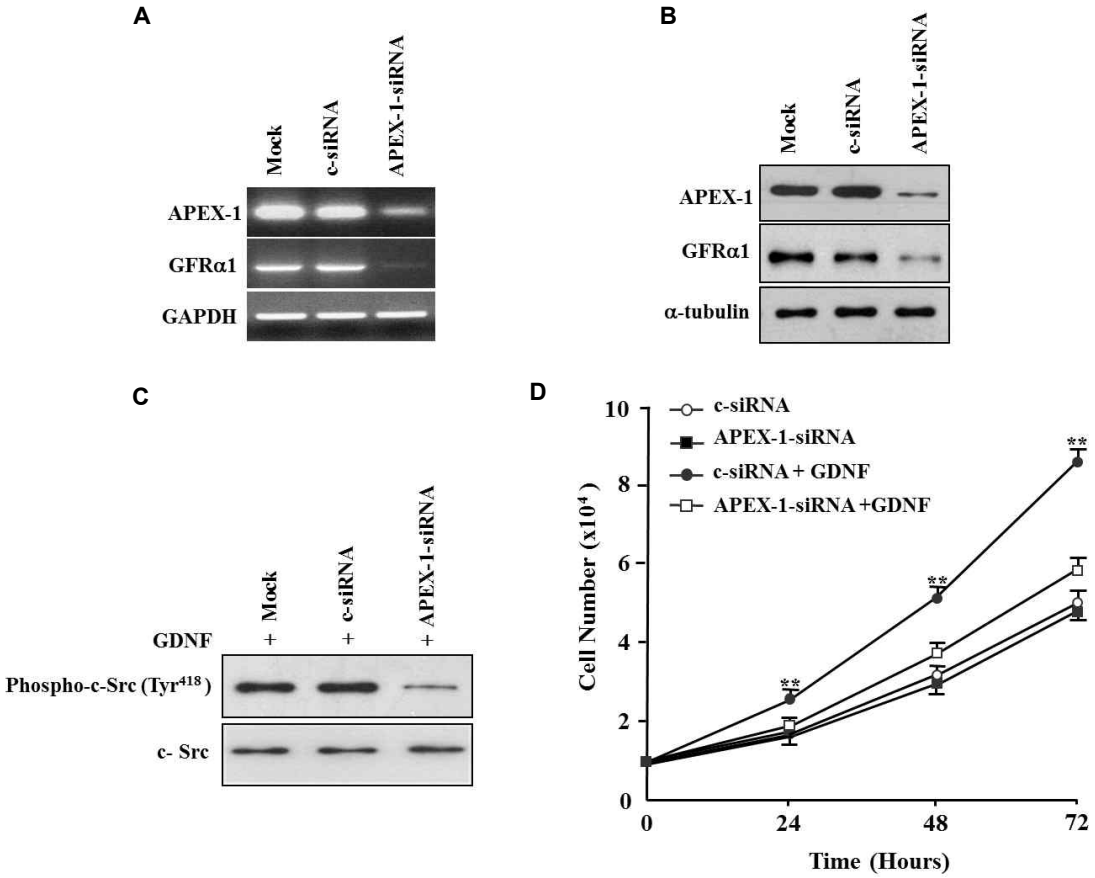

Fig. 4. Reduced APEX-1 associated with the suppressed cell proliferation in Ad-APEX-1 infected GM00637 cells. (A) The Ad-LacZ or Ad-APEX-1 infected GM00637 cells were transfected with the mock, control siRNA (c-siRNA) or APEX-1 siRNA (Ref-1-siRNA). 48 hr after transfection, the total RNA was extracted from the cells and analyzed by semiquantative RT-PCR using the *APEX-1*-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-APEX-1, anti-GFRα1 or anti-α-tubulin antibodies. (C) The Ad-LacZ or Ad-APEX-1 infected GM00637 cells were transfected with the mock, control siRNA or APEX-1 siRNA and incubated with GDNF (10 ng/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-APEX-1 infected GM00637 cells were transfected with the mock, control siRNA or APEX-1 siRNA and incubated with GDNF (10 ng/ml) for up to 72 hr. The number of cells was determined by counting the cells every 24 hr after GDNF treatment. Each value is a mean ± SD from three separate experiments. The asterisk indicates significantly different from the APEX-1-siRNA+GDNF at $p<0.01$.

APEX-1-infected cells treated with the APEX-1 siRNA. The cell proliferation experiments confirmed that the transfection of APEX-1 siRNA strongly reduces the level of cell proliferation in response to GDNF compared with the control siRNA transfection (Fig. 4D).

Discussion

Ad-APEX-1 (adenovirus encoding a human APEX-1)-infected GM00637 human fibroblast cells were used in this study to examine the effect of APEX-1 on gene expression. The results showed that APEX-1 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 APEX-1 expressing cells, as determined by its phosphorylation. Moreover, it was found that GDNF could stimulate cell proliferation in the APEX-1 expressing cells, as measured by counting the number of cells. APEX-1 specific RNA experiments demonstrated that the downregulation of APEX-1 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 APEX-1.

The GDNF was originally characterized as a potent neurotropic factor specific for the survival and differentiation of the midbrain dopaminergic neurons [12]. 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 [15]. Currently, four GFRα proteins, GFR α1, 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 [11]. 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) [1]. This study demonstrated the functional involvement of APEX-1 in the GDNF/GFRα signal pathway. The induction of GFRα1 correlated with the initiation of signaling downstream of the GDNF in the APEX-1 expressing cells. Src was phosphorylated by GDNF in the APEX-1-expressing cells. This agrees with a recent report showing that GDNF triggers Src-family kinase activation through GFRα1 independently of Ret [17]. This suggests that APEX-1 can trigger the GDNF/GFRα signal pathway indicating that APEX-1 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 [2]. 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 [13]. In addition, the pancreatic cancer cell line contained both GFRα1 and Ret and GDNF increased the invasive capacity of human pancreatic cancer cell lines [19]. 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 [8]. Interestingly, a significant increase in APEX-1 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 [3, 14, 15, 24]. A higher APEX-1 expression level was also reported to be associated with tumor progression [6]. Therefore, the APEX-1-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 APEX-1 expression is present in all somatic tissues [21]. The presence of widespread and high level of APEX-1 expression during development is expected to play an important role in embryogenesis. *APEX-1* null mice exhibits die during the embryonic stage, which results from a developmental defect [23]. The phenotype of embryonic death observed in the *APEX-1*-/- mice may be a consequence of defective DNA repair as well as inappropriate gene regulation whose expression is dependent on APEX-1. This study demonstrated that a defect in APEX-1 expression by siRNA suppressed GFRα1 expression and the GDNF responsiveness. Mice lacking GDNF [16, 18], and GFRα [4] 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 *APEX-1* null mice are embryonic lethal, one may speculate that arising from APEX-1 functional defect in APEX-1-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 APEX-1. It was also demonstrated that APEX-1 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 APEX-1 in normal development and cell proliferation mediated through GDNF /GFRα signaling.

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초록 : APEX-1은 GDNF/ GFRα1 시그널을 통해 세포증식을 조절한다

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APEX-1 (인간 apyrimidinic / apurinic 효소)은 염기성 사이트 및 DNA단일 가닥 결손으로 손상된 DNA을 복구 할 수 있는 다기능 단백질이다. 또한 APEX-1은 많은 전사 인자들의 redox-modifying factor (산화 환원 수정 요소)로서의 역할을 한다고 알려져 있다. 이런 APEX-1의 전사 타겟을 동정하는 것은 APEX-1의 다양한 세포 내 작용 메커니즘을 이해하는데 필수적이다. 따라서 이 논문에서는 먼저 Expression array analysis를 통해 glial cell-derived neurotropic factor receptor α1 (GFRα1)을 동정하였다. GFRα1은 glial cell-derived neurotropic factor (GDNF) family 수용체이며 APEX-1에 의해 발현이 증가된다. APEX-1이 과발현된 세포에서 GDNF처리에 의해GDNF/ GFRα1 시그널 타겟인 c-Src가 Tyr418잔기에서 인산화 됨을 관찰하였다. 또한 APEX-1이 과발현된 세포에 GDNF처리하면, 세포증식이 증가함을 보았다. 반면, APEX-1 발현을 siRNA을 이용하여 감소시키면 GFR α1 발현과 GDNF에 의한 c-Src 인산화 및 세포증식이 감소함을 확인하였다. 이상의 결과는 APEX-1은 GDNF/ GFRα1 시그널을 통해 세포 생존과 증식을 조절함을 증명하였다. 따라서 본 연구를 통해 APEX-1의 세포 증식을 조절하는 새로운 기전을 규명하였다.