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Amyloid precursor protein is involved in staurosporine induced glial differentiation of neural progenitor cells

Young-Don Kwak, Elena Choumkina, Kiminobu Sugaya *

Biomolecular Science Center, Burnett College of Biomedical Sciences, University of Central Florida, Orlando, FL 32816, USA

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Abstract

Staurosporine (STS) has been reported as not only a pro-apoptotic agent, but also a terminal differentiation inducer in several neuroblastoma cell lines. Here, we report involvement of amyloid precursor protein (APP) in a STS induced astrocytic differentiation of human neural progenitor cells (NT-2/D1). We found that STS-treated NT-2/D1 cells expressed astrocyte-specific glial fibrillary acidic protein (GFAP), aspartate transporter, and glutamate transporter-1 with a distinctive astrocytic morphology. STS treatment increased GFAP promoter activity and increased expression and secretion of APP in NT-2/D1 cell culture. Overexpressed APP enhanced GFAP promoter activity and expression of GFAP, while gene silencing of APP by RNA interference decreased GFAP expression. These results indicate involvement of APP in STS induced astrocytic differentiation of NT-2/D1 cells. Furthermore, suppression of ERK1/2 phosphorylation, which is known to regulate APP expression by a MEK1 inhibitor, PD098059, reduced both APP and GFAP expression in STS treated NT-2/D1 cells. Thus, STS may induce astrocytic differentiation of NT-2/D1 by increasing APP levels associate with activation of ERK pathway.

Keywords: Stem cells; Astrocytes; Teratocarcinoma; NT-2/D1; RNA interference; Differentiation; Alzheimer's disease; Down's syndrome

Human embryonic teratocarcinoma cells (NT-2/D1) derived from a testicular germ cell tumor have been intensively used as an experimental model for investigating neural differentiation [1–3]. Unlike post-mitotic CNS neurons or neuroblastoma, NT-2/D1 cells still possess some multipotency and distinctive developmental characteristics, which resemble the nature of neural stem cells [2]. By treating with all-*trans* retinoic acid (RA), NT-2/D1 cells are known to progressively differentiate into post-mitotic neurons in 3–5 weeks, expressing full neuronal characteristics and capable of forming functional synapses [4,5]. Recently, RA treatment has also been reported to differentiate NT-2/D1 into astrocytes (NT-2/A) [6], which express astrocyte specific markers such as glial fibrillary acidic protein (GFAP), astrocyte-specific glutamate transporter-1 (GLT-1)/excitatory amino acid transporters (EEAT)-2, and aspartate transporter (GLAST)/EAAT-1.

Staurosporine (STS), indolo (2, 3-α) carbazole, has been extensively used as a protein kinase C inhibitor [7] or apoptosis inducer in neuronal precursors [8], neurons [9], and other tumor cell lines. However, recent studies indicate STS also inhibits cell proliferation and induces neuronal and glial differentiation of murine embryonic stem cells [10], PC12 pheochromocytoma [11], and C6 glioblastoma [12]. Although the target and mechanism of the tropic effects of STS remains to be determined, STS may have properties not only as an apoptotic inducer but also as a differentiation inducer. In the current study, we try to elucidate mechanisms of STS-induced astrocytic differentiation using NT-2/D1 cells.

The amyloid precursor protein (APP) is a 695- to 770-amino acid, membrane spanning glycoprotein. To date, the cytotoxicity of Aβ peptides, generated from APP by sequential cuts with γ- and β-secretases, has been

* Corresponding author. Fax: +1 407 823 0956.
E-mail address: ksugaya@mail.ucf.edu (K. Sugaya).

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extensively studied since Aβ deposition is a major pathophysiology of Alzheimer's disease (AD) [13]. While physiological function of APP has not been well documented, structure of APP suggests that APP might function as a receptor via G_0 binding domain [14], or as a ligand via soluble N-terminal domain [15,16]. Secreted type of APP (sAPP) promoted neurite outgrowth of primary neuronal cultured cells [15] as well as proliferation of neural stem cells [16]. Overexpression of APP reduced the number of apoptotic neurons deprived of NGF in dorsal root ganglion [17], which may occur through p38 MAPK-dependent phosphorylation and activation of myocyte enhancer factor-2 [18]. These results indicate that APP has properties of growth and anti-apoptotic factors. Here, we demonstrate that APP is playing an important role in STS-induced astrocytic differentiation of NT-2/D1 cells.

Materials and methods

Reagents and antibodies. STS (Sigma), PD098059 (Sigma), and SB239060 (Calbiochem) were dissolved in dimethyl sulfoxide and stored at −80 °C until use. Primary antibodies: rabbit anti-GFAP antibody (Promega); mouse anti-APP antibody (22C11) (Chemicon); mouse anti-ERK antibody (BD Transduction Laboratory); and mouse anti-phospho-ERK1/2 (pT202/pY204) antibody (BD Transduction Laboratory. Secondary antibodies: anti-mouse IgG and anti-rabbit IgG horseradish peroxidase-conjugated antibodies (Jackson Immunoresearch Laboratory) were used.

Cell culture. The NT-2/D1 cells were seeded (5×10^6 cells per 150 mm petri dish) in Dulbecco's modified Eagle's medium with F-12 (DMEM/F-12; Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Novacell), 1% antibiotic-antimycotic (Invitrogen), and 4 mM glutamine (Invitrogen), and maintained in a humidified atmosphere of 5% CO_2/95% air at 37 °C. The cells were passed twice a week by short exposure to trypsin/EDTA (Invitrogen). For the experiments, 1×10^6 NT-2/D1 cells were plated in a six-well tissue culture plate and subsequently treated with 10 or 40 nM STS. Cells were rinsed twice in ice-cold phosphate-buffered saline (PBS, pH7.4) and then mRNA and protein samples were extracted for further analysis. Enhanced green fluorescent protein (EGFP) expression in the cells were detected as green fluorescence signals under a microscope (Leica, model DMRB) after fixing with 4% paraformaldehyde.

siRNA preparation. Human APP695 (GenBank Accession No. A33292) mRNA target sequences were designed using Ambion target finder software. The mRNA target sequences of human APP695 was 5′-AATCTTTGGAACAGGAAGCAG-3′ (1094–1114). siRNA PCR products were synthesized using a Silencer™ Express kit (Ambion). Then, silencing efficacy was determined using RT-PCR. As a control, we prepared EGFP siRNA PCR products (Accession No. U55763, target sequence: 106–127) as follows. In the first round PCR, hU6 promoter (GenBank Accession No. M14486, gene sequence 64–355) and 27 nt of U6 5′-coding sequence was amplified with a primer set (U6-F: 5′-TCTTTGG AATTCAAGGTCGGGCAGGAAGAGGGCCTA-3′, U6-R: 5′-CGCG GATCCTAGTATATGTGCTGCCGAAGC-3′) using plasmid pUC-hU6 [19] as a template. Simultaneously hairpin-siRNA for EGFP gene consists of 21 nt sense strand of siRNA, 9 nt spacer and 21 nt antisense strand of siRNA was generated by PCR using another primer set (siEGFP-F: 5′-C GC GGA TCC GGC GAT GCC ACC TAC GGC AAG CTC GAG ATC-3′, siEGFP-R: 5′-GCT CTA GAG GCG ATG CCA CCT ACG GCA AGG ATC TCG AGC T-3′). After 100-times dilution, first round PCR products were mixed and used as template for the second round PCR. Since 5′-end of siEGFP-F primer contains complementary sequence to 3′-end of hU6 promoter region, the second round PCR using U6-F primer and siEGFP-R primer produced hU6-siEGFP. The first and second round PCR condition were both pre-incubation at 95 °C for 5 min; 35 cycles of 95 °C for 40 s, 55 °C for 30 s, and 72 °C for 30 s; and post-extension at 72 °C for 5 min. To produce PCR fragment containing hU6 promoter with a random sequence for using as a nonspecific siRNA in the cells, we used a primer set U6-F and U6-R1 (5′-AAA AAT TCT AGA TGT AAA AAT AGT GTT GTG TGC CTA GGA TAT GTG CTG CCG AAG CGA GCA C-3′) using plasmid pUC-hU6 as a template.

Transfection. Transient transfection of pEGFAP-C1 (BD Clontech), pCEP-APP-GFP, pCEP-APP695 (kindly provided by Dr. Beth Ostaszewski, Harvard Medical School), pGFAP-GFP-S65T (kindly provided by Dr. Albee Messing, University of Wisconsin Madison), and siRNA PCR fragments (U6-random, siGFP and siAPP) was performed with Lipofectamine™ 2000 (Invitrogen) on subconfluent NT-2/D1 cells in a six-well culture plate, according to the manufacturer's protocol. Transfection efficiency in NT-2/D1 cell was determined by transfection of 0.5 μg of pEGFP-C1, and it was generally around 80%.

RT-PCR analysis. Total RNA was extracted from the cells with Trizol reagent (Invitrogen) according to the manufacturer's protocol. One microgram of the total RNA was reverse-transcribed (RT) and amplified by the SuperScript™ ONE-STEP™ RT-PCR system (Invitrogen) with the following primers: GFAP (+) 5′-AAGCAGTCTACCCACCTCAG-3′, (−) 5′-ATCCCTCCCAGCACCTCATC-3′; APP (+) 5′-CTTGAGT AAACTTTGGGACATGGCGCTGC-3′, (−) 5′-GAACCCTACGAAG AAGCC-3′; GLT-1 (+) 5′-GACAGTCATCTTGGCTCAGA-3′, (−) 5′-A ATCCACCATCAGCTTGGCC-3′; GLAST (+) 5′-CTGCTCACAGTC A CCGCTGT-3′, (−) 5′-AGCACGAATCTGGTGACGCG-3′; EGFP (+) 5′-CAAGGACGACGGCAACTACAAGAC-3′, (−) 5′-GCGGACT GGGTGCTCAGGTAGTGGT-3′; β-actin (+) 5′-GACAGGATGCAG A AGGAGAT-3′, (−) 5′-TTGCTGATCCAC ATCTGCTG-3′. RT-PCR condition was a reverse transcription at 55 °C for 30 min; a pre-denaturation at 94 °C for 2 min; 25–32 cycles of 94 °C for 15 s; 55–56 °C for 30 s; and 72 °C for 30 s; and a post-extension at 72 °C for 5 min. Cycle number was adjusted in order to keep the PCR amplification in a log-phase for semi-quantization. Ten microliters of the reaction was separated in a 2% E-gel (Invitrogen).

Immunoprecipitation and Western blot analysis. Protein samples were prepared by lysing the cells using an ice-cold lysis buffer consisting of 1% NP40, 150 mM NaCl, 50 mM Tris, pH 8.0, and Complete Protease Inhibitor Tablets (Boehringer). The protein concentration of each sample was measured by Bio-Rad protein assay (Bio-Rad). Lysates were immunoprecipitated with an antibody against APP, GFAP, and ERK molecules using protein A-Sepharose (Amersham Bioscience). Then, precipitates and, in some cases, cell lysates were heated at 70 °C for 10 min in a sample loading buffer and separated on NuPAGE™ 4–12 % Bis-Tis Gel (Invitrogen) for 45 min at 200 V and transferred to a PVDF membrane (30 V, 60 min). Membranes were blocked with 5% skim milk in PBS for 1 h at room temperature and probed at 4 °C overnight with primary antibody in 5% skim milk. The membranes were washed three times for 5 min each with PBS containing 0.05% Tween 20 (pH7.4) and then incubated with horseradish peroxidase-conjugated secondary antibodies in 5% skim milk for 2 h at RT. After three times washing with PBS containing 0.05% Tween 20, immunoreactive bands were visualized by using ECL plus (Amersham Bioscience) chemiluminescence reagent.

Image analysis. Gel or Western blot images were captured by KODAK Image Station 2000MM. Optical density of the target bands was analyzed using ImageJ (Ver 4.1, NIH) and expressed in means ± SD from experiments performed in triplicate.

Results

Induction of glial cell like morphological change of NT-2/D1 cells by STS

As a further test for function of STS as a differentiation inducer, we analyzed morphological changes after STS treatment. NT-2/D1 cells grew as a monolayer culture of

neuroepithelial cells with a high nuclear to cytoplasmic ratio and prominent nucleoli resembling embryonic carcinoma cells (Fig. 1A-a). Morphological changes started right after day 1 of treatment with 40 nM STS (Fig. 1A-b). Dramatic change of morphology occurred after 7 day treatment of 40 nM STS (Fig. 1A-c) and these changes continued during 14 day treatment of STS (Fig. 1A-d). Based on morphological features, protoplasmic and polygonal irregular star-like cells, STS treated cells showed distinctive astrocytic cell morphology (Fig. 1A-c and d), indicating that STS treatment induced astrocytic differentiation of NT-2/D1 cells.

GFAP expression in STS-treated NT-2/D1 cells

Although physiological function of GFAP during astrocytic differentiation is still unclear, GFAP has been used as a typical marker for astrocytic differentiation [20,21]. To characterize the morphological changes in NT-2/D1 after STS treatment, we analyzed GFAP expression in these cells using RT-PCR (Fig. 1B). Levels of GFAP mRNA were increased by STS-treatment compared to the basal level of GFAP mRNA in control cells with time-dependent manner up to 24 h. These cells also expressed astrocyte-specific glutamate transporter-1 (GLT-1)/excitatory amino acid transporters (EEAT)-2 and aspartate transporter (GLAST)/EAAT-1 (Fig. 1C).

We also confirmed GFAP promoter activation by STS treatment using NT-2/D1 transfected with GFAP promoter driven GFP plasmid vector (pGFAP-GFP-S65T) as a reporter system. As shown in Figs. 1D and E, 10 nM STS treatment induced both mRNA and protein expression (green fluorescence) of GFP. These results indicate that STS induces astrocytic differentiation of NT-2/D1 cells.

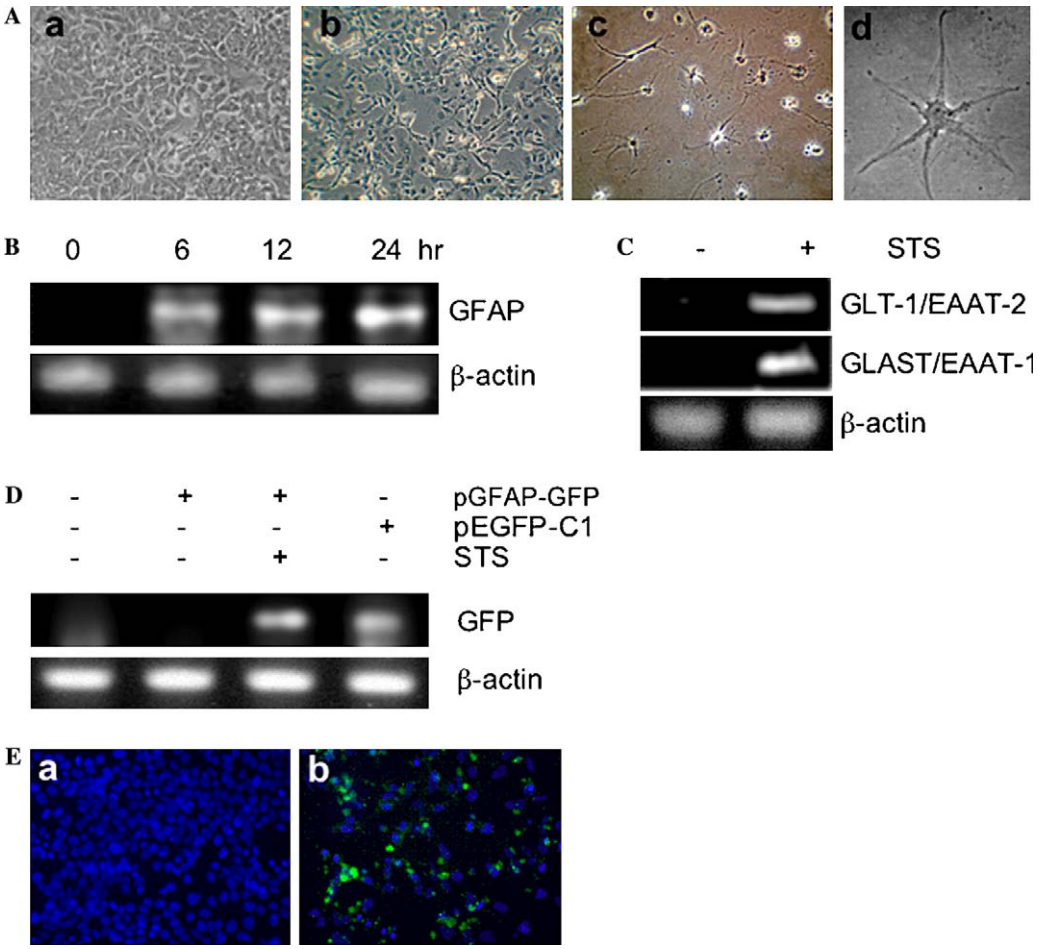

Fig. 1. Morphological changes of NT-2/D1 cells and induction of astrocyte specific gene expression by STS. (A) NT-2/D1 cells grew as a monolayer culture resembling embryonic carcinoma cells (a, 100×). Morphological changes started right after day 1 of treatment with 40 nM STS (b, 100×). Astrocyte like morphology was observed after 7 days (c, 100×) and 14 days (d, 400×) treatment with STS. (B) RT-PCR analysis of NT-2/D1 cells treated with 10 nM STS for 0, 6, 12, and 24 h shows time-dependent increase of GFAP gene expression. Expression of β-actin was used for an internal control. (C) RT-PCR analysis shows treatment with 10 nM STS for 24 h induced astrocyte specific glutamate transporter expressions (GLT-1/EAAT-2 and GLAST/EAAT-1) in NT-2/D1 cells. (D) RT-PCR analysis of mRNA of GFP transcribed by GFAP promoter in NT-2/D1 cells transfected with pGFAP-GFP-S65T as a reporter system. After 2 days, the cells treated with 10 nM STS for 24 h expressed GFP gene. (E) Fluorescence image of GFP in NT-2/D1 cells transfected with pGFAP-GFP-S65T before 10 nM STS treatment (a) and 24 h after the treatment (b).

Increased gene expression level of APP during STS-induced astrocytic differentiation

Both mRNA and protein expression of APP in NT-2/D1 time-dependently increased after 10 nM STS treatment (Figs. 2A and B). In addition, concentration of sAPP also time-dependently increased in the media of NT-2/D1 cell culture (Fig. 2B). Then, the increased catabolism of APP showed the correlation with the GFAP expression in the NT-2/D1 cells (Fig. 2B). This result suggests that STS-induced APP expression may have an important role in astrocytic differentiation of NT-2/D1 cells.

sAPP induced GFAP expression in NT-2/D1 cells

We already reported STS enhanced gene expression of GFP by stimulating GFAP promoter activity (Figs. 1D and E). However, it was not clear whether STS had a direct affect on the GFAP promoter or GFAP promoter activity is regulated by other factors, such as APP, which is induced by STS treatment. Thus, we examined GFAP promoter activity by assessing GFP expression using NT-2/D1 cells transfected with GFAP promoter driven GFP expression vector (pGFAP-GFP-S65T) (Fig. 3). When APP695 expression vectors (pCEP-APP695) were overexpressed in reporter vector transfected cells, gene expression of GFP was detected. Therefore, these results suggest that APP is involved in astrogliogenesis of NT-2/D1 cells in STS treated condition.

Gene silencing of APP expression suppresses GFAP expression

Recently small interference RNA (siRNA) has been extensively used for elucidating specific functions of the gene of interest [22]. To confirm the involvement of APP in STS-induced astrocytic differentiation, we investigated whether STS function in NT-2/D1 is abolished by knocking down the gene expression of APP using siRNA. We constructed specific siRNA PCR products (siAPP-PCR), which specifically recognize APP. Small interference APP-PCR significantly decreased protein expression of APP-GFP fusion protein in NT-2/D1 cells transfected with pCMV-APP-GFP (Fig. 4A-d). Control PCR products (U6-random sequence PCR) did not inhibit APP-GFP fusion protein expressions.

Small interference APP PCR also showed significantly suppressed APP and GFAP gene expressions in NT-2/D1 cells treated with STS, while control siRNA PCR products (siGFP-PCR), which recognize GFP, did not suppress APP and GFAP expression (Figs. 4B and C). These findings suggest that APP is a crucial molecule for astrocytic differentiation of NT-2/D1 cells induced by STS treatment.

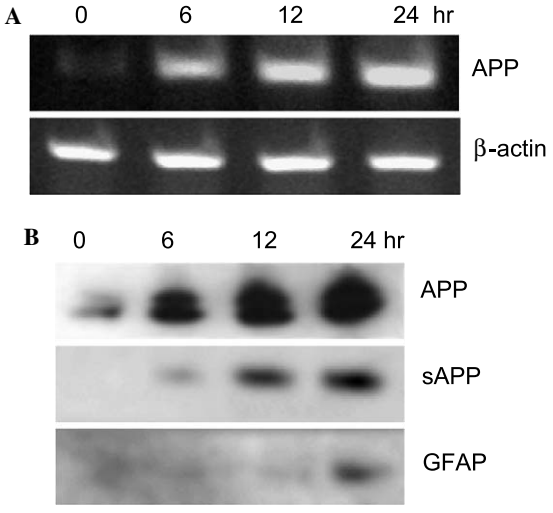

Fig. 2. Time-dependent increase of APP expression after STS treatment. (A) RT-PCR analysis of APP gene expression in NT-2/D1 cells treated with STS. (B) Western blot analysis of APP, sAPP, and GFAP protein expression in 10 nM NT-2/D1 cells treated with 10 nM STS. Samples for APP and GFAP were isolated from the cell lysates; sample for sAPP was isolated from the NT-2/D1 cell culture media.

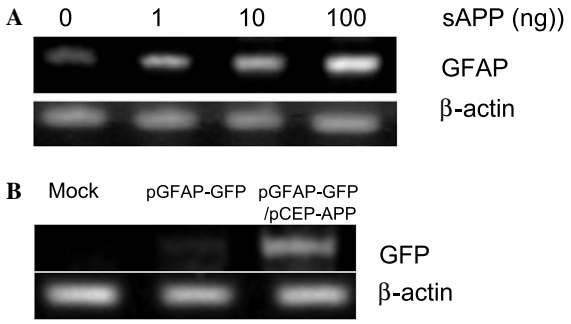

Fig. 3. Increased GFAP expression by treatment with recombinant sAPP or overexpression of APP gene. (A) RT-PCR analysis of mRNA of GFAP gene expression analysis in NT-2/D1 cells treated with various amount (0, 1, 10, and 100 ng) of recombinant APP shows dose-dependent increase of GFAP gene expression. Expression of β-actin was examined as a loading control. (B) RT-PCR analysis of mRNA of GFP transcribed by GFAP promoter in NT-2/D1 cells transfected with pGFAP-GFP-S65T (1 μg each) as a reporter system. Expression of GFP mRNA was observed only after co-transfection with APP expression vector (pCEP-APP695).

Small interference APP PCR also showed significantly suppressed APP and GFAP gene expressions in NT-2/D1 cells treated with STS, while control siRNA PCR products (siGFP-PCR), which recognize GFP, did not suppress APP and GFAP expression (Figs. 4B and C). These findings suggest that APP is a crucial molecule for astrocytic differentiation of NT-2/D1 cells induced by STS treatment.

STS activate ERK pathway, which regulates APP expression

Although STS is known to be a PKC inhibitor [7], it is also reported to activate mitogen-activated protein kinase (MAPK) signal transduction [23], which is a major pathway responsible for APP catabolism [24]. Treatment of NT-2/D1 cells with 10 nM STS time-dependently increased phosphorylation of ERK1/2 (p44/42 MAP kinases) after 45 min of incubation (Fig. 5A). To further test the role of ERK signaling in STS-induced APP expression, we pretreated NT-2/D1 cells with PD098059 [25], a selective inhibitor of MEK1 or SB203580 [26], a selective inhibitor of p38 MAPK for 30 min prior to a 10 nM STS treatment with PD098059 or SB203580 for 24 h. PD098059 inhibited STS-induced APP (Fig. 5B) and GFAP (Fig. 5C) production at a concentration of 20 μM or greater, whereas SB203580 did not significantly alter APP and GFAP

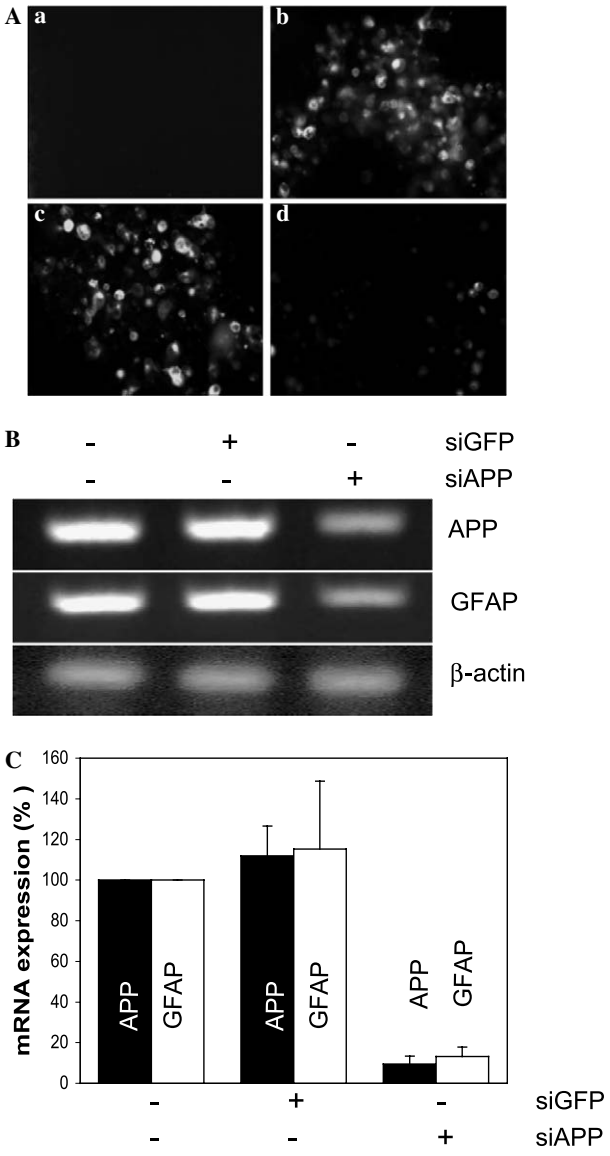

Fig. 4. Assessment of APP functions by RNAi for APP in NT-2/D1 cells. (A) A mock-transfected NT-2/D1 cells, as a negative control, show no fluorescent signal (a). NT-2/D1 cells transfected with 1 μg of pEGFP-C1, as a positive control, show significant level of green fluorescent signals (b). NT-2/D1 cells in both c and d received transfection of 1 μg of pCEP-APP-GFP to express APP-GFP fusion protein. Co-transfection of 1 μg of U6-random PCR products expressing random sequence does not suppress expression of the APP-GFP fusion protein (c). Co-transfection of 1 μg of siRNA PCR products targeting APP significantly suppressed expression of the APP-GFP fusion protein (d). Experiments were repeated three times with similar results. (B) Silencing of APP reduced GFAP expression stimulated by 10 nM STS in NT-2/D1 cells. Gene expression levels of APP and GFAP were assessed by RT-PCR in NT-2/D1 cells treated with 10 nM STS were transfected with each 1 μg of siRNA of PCR products targeting GFP (siGFP) or APP (siAPP) for 2 days. Transfection of siAPP but not siGFP significantly reduced the expression of APP as well as GFAP mRNA levels in the NT-2/D1 cells. (C) The data (means ± SD, $n = 3$) presented are % changes of mRNA expression of APP and GFAP compared to the control, NT-2/D1 cells only treated with 10 nM STS, as 100%.

production. Similarly, mRNA expression of APP also was inhibited by PD098059 at a concentration of 20 μM or greater (Figs. 5D and E). In addition, mRNA expression

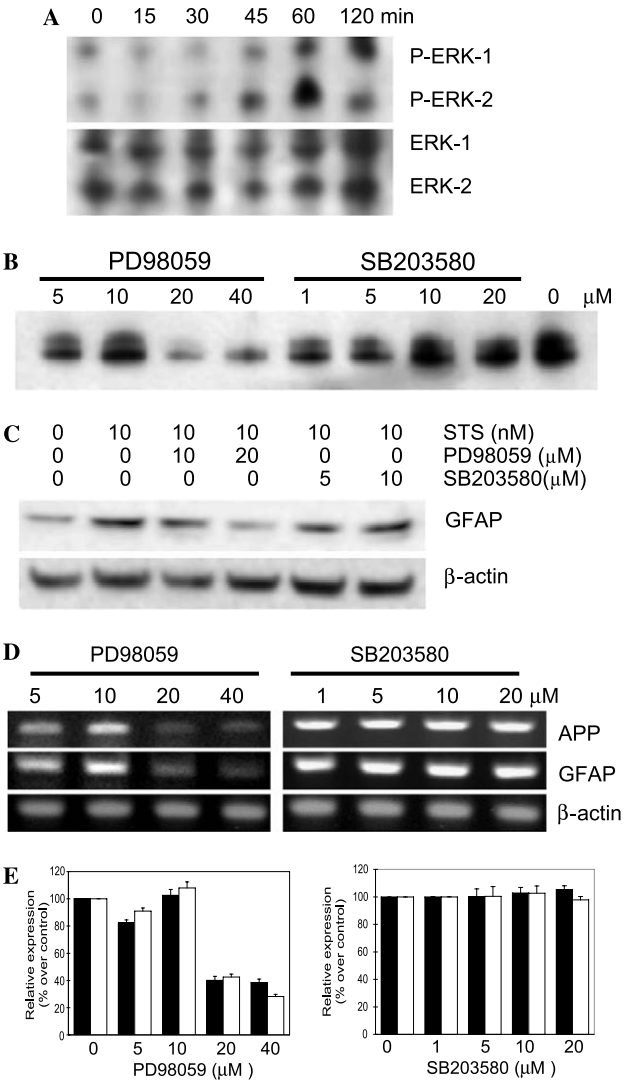

Fig. 5. The involvement of ERK1/2 pathway in STS-induced astrocytic differentiation of NT-2/D1. (A) NT-2/D1 cells were plated at a density of 1×10^6 cells and then treated with 10 nM STS for indicated time period. Cell lysates were subjected to immunoprecipitation with anti-human-pan-ERK Ab. Parallel blots were probed with anti-phospho-p44/42 ERK Ab (P-ERK-1/2) and anti-pan-ERK Ab (ERK-1/2). Phosphorylation of ERK1/2 increased progressively after 45 min. (B) PD098059, a selective MEK1 inhibitor, decreased APP expression activated by 10 nM STS in NT-2/D1 cells. While SB203580, a selective p38 MAPK inhibitor, did not affect APP expression. (C) PD98059, decreased GFAP expression activated by 10 nM STS in NT-2/D1 cells, while SB203580 did not affect GFAP expression. (D) PD98059, decreased GFAP, and APP gene expression activated by 10 nM STS in NT-2/D1 cells, while SB203580 did not affect the gene expression. Gene expressions of APP and GFAP were examined by RT-PCR in NT-2/D1 cells pretreated with various concentrations of PD098059 or SB203580 for 30 min and exposed to 10 nM STS for 24 h. (E) Semi quantitative image analysis of gene expression data from (D). The data (means ± SD, $n = 3$) presented are % increase of mRNA expression of APP and GFAP compared to the control as 100%.

levels of GFAP decreased according to the decrease of APP expression (Figs. 5D and E). These results indicate that STS-induced astrocytic differentiation associated with increased APP and GFAP expressions may be mediated by ERK1/2 signaling pathway not by p38 MAPK pathway.

Discussion

NT-2/D1 cells have been thought to exclusively differentiate into pure neurons as a neuronal progenitor. However, recent studies show astrocytic differentiation of NT-2/D1 cells by RA treatment [1,6], indicating that it is a good in vitro model system for analyzing regulation of neural differentiation. In the current study, we report that STS induces astrocytic differentiation of NT-2/D1 by increasing APP expression via activation of ERK1/2 signaling cascade.

Typical astrocytic morphology appeared in the culture after 7 days of treatment with STS and more than 90% of cells showed this feature by week 2. These cells expressed high levels of not only GFAP, a typical astrocyte marker but also astrocyte-specific glutamate transporters such as GLAST/EAAT1 and GLT1-EAAT2, which are used for removing excessive glutamate to maintain a physiological level of extracellular glutamate concentration [27], indicating that STS differentiated NT-2/D1 cells into astrocytes. Furthermore, STS treatment increased green fluorescent in NT-2/D1 transfected with reporter vector (pGFAP-GFP-S65T), indicating activation of GFAP promoter by STS.

Apoptosis is closely associated with differentiation during development of the neuronal system [28]. Recent reports also show STS, a potent apoptosis inducer, causes early neural stem cell like differentiation of embryonic stem cells [10]. Although the mechanism is not clear, STS is also reported to induce astrocytic phenotypes in C6 glial cells [12]. Thus apoptotic cascade triggered by STS may be involved in the astrocytic differentiation.

We found that APP expression, which is reported to be increased during apoptosis [29], increased in NT-2/D1 cell culture after STS treatment. Although to date, most of APP studies have been focused on neurotoxicity of Aβ, several lines of evidence suggest that APP affects mechanisms of anti-apoptosis and neurite outgrowth [15,18]. Thus, the increased expression levels of APP may be a compensation mechanism of the apoptotic condition created by STS treatment. Down's syndrome (DS) is associated with a high incidence of AD with massive gliosis [30,31]. Recently Bahn et al. [32] demonstrated that neurospheres derived from DS patients almost exclusively differentiated into GFAP positive cells. Since DS patients have trisomy of chromosome 21, where the APP gene is localized, we associate these incidents to an overexpression of APP and speculate that STS-induced APP expression might play a crucial role in glial differentiation of NT-2/D1 cells.

In this study, knocking down APP expression of NT-2/D1 cells using RNA interference (RNAi) against APP reduced GFAP expression induced by STS treatment. Although STS may induce astrocytic differentiation of NT-2/D1 through various signaling mechanisms, this result suggests that at least APP is involved in the induction of GFAP expression during STS-induced glial differentiation.

MEK1 inhibitor (PD098059), which reduces phosphorylation of ERK1/2, dose-dependently reduced expression of APP and GFAP. Since APP expression is regulated by phosphorylation of ERK1/2 [33] and STS increases phosphorylation of ERK1/2 [23], STS may increase APP expression level by ERK1/2 phosphorylation. On the other hand, p38 MAP kinase inhibitor (SB203580) did not show any significant effect on APP and GFAP expression. These results indicate that STS may induce GFAP expression through the upregulation of APP by increasing ERK1/2 phosphorylation.

It is not clear how adult neurogenesis is essential for normal cognitive function in aging. Although the rate of endogenous neuroregeneration in the adult brain may be minimal, in the long run, pathologically-altered APP metabolism in AD or DS causes a defect in neurogenesis and significantly harms normal brain function. This fact could also prevent successful neuroreplacement therapy for AD using NSC by shifting the differentiation pattern of the transplanted cells into glial cells rather than into neurons. Thus, in order to use stem cell transplantation as a potential strategic intervention therapy for AD or DC. Regulation of APP levels and/or modifications of the APP signal pathways within the cells may need to be developed along with a better understanding of how the mechanisms of APP function in neural stem cell biology.

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