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ORIGINAL PAPER

Effects of Nerve Growth Factor and Basic Fibroblast Growth Factor Promote Human Dental Pulp Stem Cells to Neural Differentiation

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Abstract Dental pulp stem cells (DPSCs) were the most widely used seed cells in the field of neural regeneration and bone tissue engineering, due to their easily isolation, lack of ethical controversy, low immunogenicity and low rates of transplantation rejection. The purpose of this study was to investigate the role of basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) on neural differentiation of DPSCs in vitro. DPSCs were cultured in neural differentiation medium containing NGF and bFGF alone or combination for 7 days. Then neural genes and protein markers were analyzed using western blot and RT-PCR. Our study revealed that bFGF and NGF increased neural differentiation of DPSCs synergistically, compared with bFGF and NGF alone. The levels of Nestin, MAP-2, βIII-tubulin and GFAP were the most highest in the DPSCs + bFGF + NGF group. Our results suggested that bFGF and NGF signifiantly up-regulated the levels of Sirt1. After treatment with Sirt1 inhibitor, western blot, RT-PCR and immunofluorescence staining showed that neural genes and protein markers had markedly decreased. Additionally, the ERK and AKT signaling pathway played a key role in the neural differentiation of DPSCs stimulated with bFGF + NGF. These results suggested that manipulation of the ERK and AKT signaling pathway may be associated with the differentiation of bFGF and NGF treated DPSCs. Our date provided theoretical basis for DPSCs to treat neurological diseases and repair neuronal damage.

Keywords Dental pulp stem cells (DPSCs) · Neural differentiation · Silent information regulator protein 1 (Sirt1) · Basic fibroblast growth factor (bFGF) · Nerve growth factor (NGF)

Introduction

The treatment of neurological diseases caused by irreversible neuronal cell damage, loss of neuronal cells or necrosis, is a world-wide problem [1]. In recent years, many attempts have been made to address this problem by cell transplantation and gene therapy. Dental pulp stem cells (DPSCs) are multipotent stem cells. DPSCs in regenerative medicine is anticipated, due to highly proliferative cells capable of self-renewal and be induced to differentiate into several lineages including chondrogenic, adipogenic, neurogenic, osteogenic and myogenic [2, 3]. DPSCs were the most widely used seed cells in the field of neural regeneration and bone tissue engineering, due to their easily isolation, lack of ethical controversy, low immunogenicity and low rates of transplantation rejection [4–6]. Moreover, numerous researches have demonstrated that DPSCs are able to differentiate into neuron-like cells in vitro via genetic manipulation, where various factors and chemical agents are adopted to induce DPSCs differentiation into neuron-like cells.

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Basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) are powerful mitogens that improve the nutrition of neural stem cells and precursor cells present in the mature nervous system [7]. bFGF is often considered as a growth factor and differentiation inducer within the stem cell research field. Furthermore, bFGF has previously been demonstrated to maintain mesenchymal stem cells (MSCs) differentiation potential and increase their telomere length in various culture systems [8, 9]. bFGF has been reported to be a potent mitogenic factor for neural stem and progenitor cells both *in vitro* and *in vivo*. Studies have showed that cultured hippocampal neural progenitor cells divide only in response to bFGF [10]. NGF is a homodimeric peptide. NGF can regulate cell growth and promote neural differentiation by supporting the survival and growth of neural cells in the nervous system. Moreover, NGF shows nerve injury healing ability in clinical therapy [11]. NGF can induce bone marrow MSCs differentiation into neural cells, via generating neuropeptide signals and receptors [12]. In some reports, DPSCs showed better neural stem cell properties than bone marrow derived MSCs [13]. It has been shown that human dental pulp cells expressed and secreted NGF [6]. NGF combinated other neurotrophic factors were added into serum-free low glucose DMEM/F-12 medium to induce the neurogenic differentiation of DPSCs [14].

Silent information regulator protein 1 (Sirt1) is most homologous to the founding member of the Sir2 family from yeast [15]. It is a nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase and a class III histone deacetylase participated in immune reactions, inflammation, cell differentiation, cell survival and cell metabolism [16–20]. Recent studies have demonstrated that Sirt1 activation plays an important role against age-related diseases because because its neuroprotective effects correlated with its functions in metabolism, stress resistance, and genomic stability [21, 22]. There is increasing evidence that Sirt1 activation has an important effect on neuronal architecture by stimulating axon elongation and neurite outgrowth [23–25]. Cytoplasmic Sirt1 down-regulated mTOR and stimulated neurite outgrowth, which indicated the role of Sirt1 in neuronal differentiation and the structural features of neuronal cells [24, 25]. Sirt1 activation has been reported to induce the neuronal differentiation and has beneficial effects on neurodegenerative diseases [22, 23, 26]. Previous reports have been suggested to promote neurite outgrowth and the subcellular localization of this deacetylase is critical for its function [22]. Recently, it was shown that MSCs could be effectively differentiated into neurons by Sirt1 activator treatment combined with neuronal induction media [27]. These data suggest that Sirt1 may play a key role in the induction of neuronal differentiation.

In present study, we explored the possibility and value of using NGF and bFGF in combination to promote neuronal differentiation of human DPSCs. We also analyzed the role of the Sirt1 for the first time in vitro, and our aim is to provide theoretical basis for further research in vivo.

Materials and Methods

Cell Cultures

Normal human impacted third molars were collected from patients 16–23 years of age (n = 9) after giving the informed consents which were approved by the Ethics Committee of the Affiliated Hospital of Nantong University. All subjects were free of carious lesions and oral infection. We isolated DPSCs by cleaning the tooth surface, cutting around the cement-enamel junction using sterilized dental fissure burs and then opening to reveal the pulp chamber. The pulp was digested in a solution of 3 mg/ml collagenase type I for 1 h at 37 °C. Single-cell suspensions were obtained by passing the digested tissues through a 70-μm cell strainer (BD Falcon). Cell suspensions of dental pulp were seeded into 25 cm^2 culture dishes and cultured in low glucose Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2. The medium was changed every 3 days. Cells were passaged at the ratio of 1:3 when they reached 85–90% confluence. The specific cell markers of DPSCs were characterized by flow cytometric analysis, with highly positive for CD29 and CD105, but negative for CD31 and CD34 [28]. All experiments were conducted on DPSCs cultured in passage 3 (P3) [3, 29].

Neurogenic Differentiation of DPSCs

To induce neurogenic differentiation, DPSCs were seeded into 24-well plates at a density of 1,000 cells/well and cultured in serum-free low glucose DMEM/F-12 medium containing 2% B27, 2% N2 (both PAA Laboratories, Coelbe, Germany), 25 ng/ml brain-derived neurotrophic factor (BDNF, R&D Systems), 100 ng/ml NGF (R&D Systems) and 25 ng/ml bFGF (R&D Systems) for 7 days. Differentiation media were changed after 3 days. Four groups contain serum-free low glucose DMEM/F-12 medium containing 2% B27, 2% N2, 25 ng/ml BDNF. Four groups were analyzed with the following stimuli added to the culture: (1) DPSCs stimulated with normal saline as a control; (2) DPSCs stimulated with NGF; (3) DPSCs stimulated with bFGF; (4) DPSCs stimulated with NGF and bFGF. Nicotinamide (NAM; 100 μM; Sigma), a Sirt1 inhibitor, was added to serum-free DMEM/F-12 medium for 4 days. Subsequent procedures were performed as above-described. Three groups contain serum-free low glucose DMEM/F-12 medium containing 2% B27, 2% N2, 25 ng/

ml BDNF. Three groups were analyzed with the following stimuli added to the culture: (1) DPSCs stimulated with normal saline as a control; (2) DPSCs stimulated with NGF + bFGF; (3) DPSCs stimulated with Sirt1 inhibitor + NGF + bFGF. The levels of neural lineage markers in the induced cells from DPSCs were assessed using western blot, RT-PCR analysis and immunofluorescence staining. Three groups contain serum-free low glucose DMEM/F-12 medium containing 2% B27, 2% N2, 25 ng/ml BDNF. Three groups were analyzed with the following stimuli added to the culture: (1) DPSCs stimulated with normal saline as a control for 7 days; (2) DPSCs stimulated with NGF + bFGF for 7 days; (3) DPSCs were incubated with 1 μM resveratrol for 12 h. DPSCs stimulated with NGF + bFGF for 7 days.

Western Blot

Cells were lysed in buffer consisting of 50 mM TRIS, 150 mM NaCl, 2% sodium dodecyl sulfate (SDS) and a protease inhibitor mixture. After centrifugation at 12,000 rpm for 12 min, protein concentrations were determined by using the Bradford assay (Bio-Rad). The resulting supernatant (50 μg protein) was subjected to SDS polyacrylamide gel electrophoresis (PAGE). The separated proteins were transferred onto polyvinylidene difluoride membranes at 350 mA for 2.5 h in a blotting apparatus (BioRAD, Calif., USA). Membranes were blocked with 5% nonfat milk and incubated with primary antibodies (1:400) at 4 °C overnight and subsequently with anti-rabbit horseradish peroxidase- conjugated secondary antibodies (1:1000) for 2 h at room temperature. Concomitantly, D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was run as a reference protein. We simply detected endogenous GAPDH with an antibody. The following primary antibodies were used: GAPDH (anti-rabbit, Santa Cruz), Nestin (anti-rabbit, Sigma), MAP-2 (anti-rabbit, Sigma), βIII-tubulin (anti-rabbit, Sigma), GFAP (anti-rabbit, Sigma), Sirt-1 (anti-rabbit, Sigma), Akt (anti-rabbit, Cell Signaling), and p-Akt (anti-rabbit, Cell Signaling), ERK (anti-rabbit, Cell Signaling) and p-ERK (anti- rabbit, Cell Signaling).

Immunofluorescence Staining

DPSCs were fixed with 4% paraformaldehyde (PFA) for 1 h at 7 days after induction, washed with PBS containing 0.1% Triton X-100 (PBST), and the cells were blocked in 1% bovine serum albumin (Sigma–Aldrich, St. Louis) for 30 min. The cells were then incubated with the one of the following primary antibodies overnight at 4 °C: Nestin (anti-rabbit, Sigma), MAP-2 (anti-rabbit, Sigma), βIII-tubulin (anti-rabbit, Sigma), GFAP (anti-rabbit, Sigma), Sirt-1 (anti-rabbit, Sigma). After washing with PBS, the cells were incubated with the following secondary antibodies for 2 h at room temperature in the dark: goat-antirabbit (cy3)-conjugated antibodies (1:300, ICN Cappel, USA), and goat-antimouse FITC-conjugated antibodies (1:300, Dako, USA). Nuclei were counterstained with DAPI (1:800, Santa Cruz). After being washed and mounted, the cells were examined with a fluorescence microscope.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total cellular RNA was isolated from cells and reverse transcribed using conventional protocols. PCR amplification was performed using the following primer sets: GAPDH 5′-TCCATGACAACTTTGGTATCG-3′, 5′-TGTAGCCAAATTCGTTGTCA-3′; GFAP 5′-GCTTCCTGGAACAG CAAAAC-3′, 5′-GGCTTCATCTGCTTCCTGTC-3′; Nestin 5′-CTC TGACCTGTCAGAAGAAT-3′, 5′-CCCACTTTCTTCCTCATCTG-3′; MAP-2 5′-CTGGGTCTACTGCCATCACTC-3′, 5′-CCCCTTTAGGCT GGTATTTGA-3′; βIII-tubulin 5′-GGGCCAAGTTCTGGGAAGTC-3′, 5′-ATCCGCTCCAGCTGCAAGT-3′; Sirt-1 5′-GGAAGCGTTTTTTTC GAGTAC-3′, 5′-CCGAATCCAAACTATAATATCTACG-3′. All the primer sequences were determined using established GenBank sequences. The primers were used to amplify the duplicate PCRs. Each sample was analyzed in triplicate and GAPDH was used as a control.

Statistical Analysis

The data are represented as mean ± standard deviation (SD) of three or more independent experiments. Statistical comparisons between groups were made using an independent t-test. P-values < 0.05 were considered statistically significant.

Results

bFGF and NGF Promoted Neural Differentiation of DPSCs

bFGF and NGF are powerful mitogens that promote the nutrition of neural stem cells and precursor cells present in the mature nervous system. To investigate the influence of bFGF and NGF on neural differentiated DPSCs, DPSCs were treated with bFGF and NGF for 7 days alone or in combination during differentiation. We examined the expressions of neuronal markers Nestin (a neural stem cell marker), MAP-2 (neurons), βIII-tubulin (a neuronal specific tubulin) and GFAP (astrocytes) to determine the neural differentiation potential of DPSCs. As shown in Fig. 1a, b,

bFGF and NGF increased neural differentiation of DPSCs synergistically, compared with bFGF and NGF alone ($P < 0.05$). When levels of neuron lineage markers including Nestin, βIII-tubulin, MAP-2 and GFAP were analyzed by RT-PCR, and Western blot, we found an increase of the markers when compared to GAPDH. Both the mRNA and protein levels increased in the presence of the differentiation factors (Fig. 1c, d).

Expression of Sirt1 Increased in DPSCs Stimulated with bFGF and NGF During Differentiation

Since Sirt1 activation has been reported to induce the neuronal differentiation and has beneficial effects in neurodegenerative diseases [27]. To determine whether bFGF and NGF have an effect on Sirt1 expression, we analyzed Sirt1 protein and mRNA levels. Compared with the control group, Sirt1 protein levels in bFGF-treated and NGF-treated groups significantly increased especially in co-treated groups (Fig. 2a, b). The mRNA changes of Sirt1 were similar to that observed in the protein levels. Sirt1 mRNA levels in co-treated groups were significantly higher compared with bFGF and NGF-treated groups (Fig. 2c, d). The Sirt1 expression was confirmed using immunofluorescence staining. The up-regulation of these proteins was observed for both bFGF-induced and NGF- induced DPSCs especially in co-treated group (Fig. 2e, f). These results suggested that bFGF and NGF signifiantly up-regulated the levels of Sirt1.

ERK and AKT Signaling were Highly Active in bFGF and NGF Induced DPSCs and Reversed by Sirt1 Inhibitor

MAPK has a significant role in the growth and differentiation of MSCs. In this study, western blot was used to

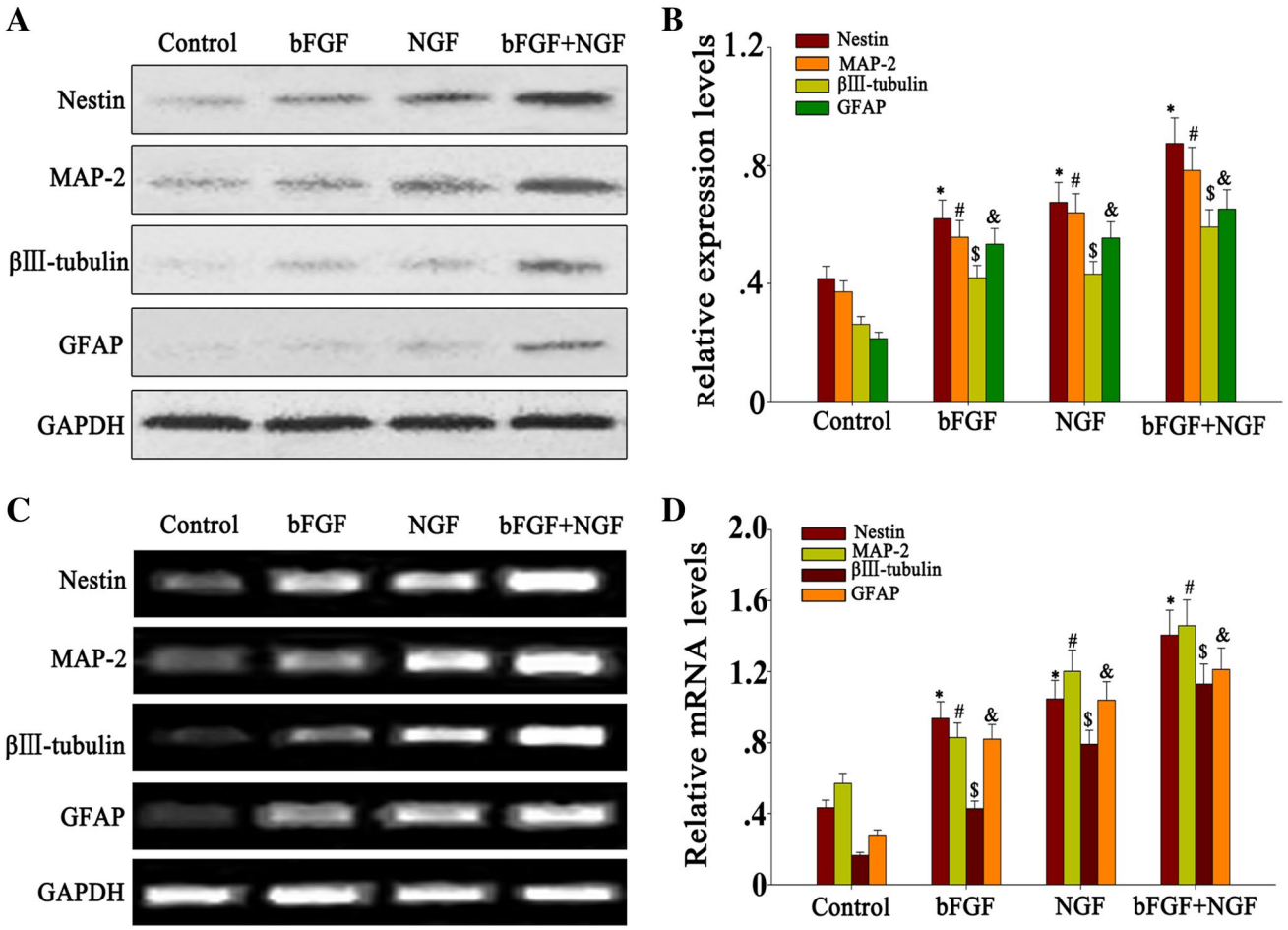

Fig. 1 bFGF and NGF promoted neural differentiation of DPSCs. **a** After treatment with bFGF and NGF alone or in combination, expressions of Nestin, MAP-2, βIII-tubulin and GFAP were analyzed by Western blot; cells in neural differentiation medium for 7 days were as control. **b** Quantification of Nestin, MAP-2, βIII-tubulin and GFAP protein levels. $*P < 0.05$. **c** Total RNA was isolated at 7 days after induction of differentiation, followed by RT-PCR analysis. **d** Quantitation of PCR products. The quantity of amplified product was analyzed by an image analyzer. $*P < 0.05$

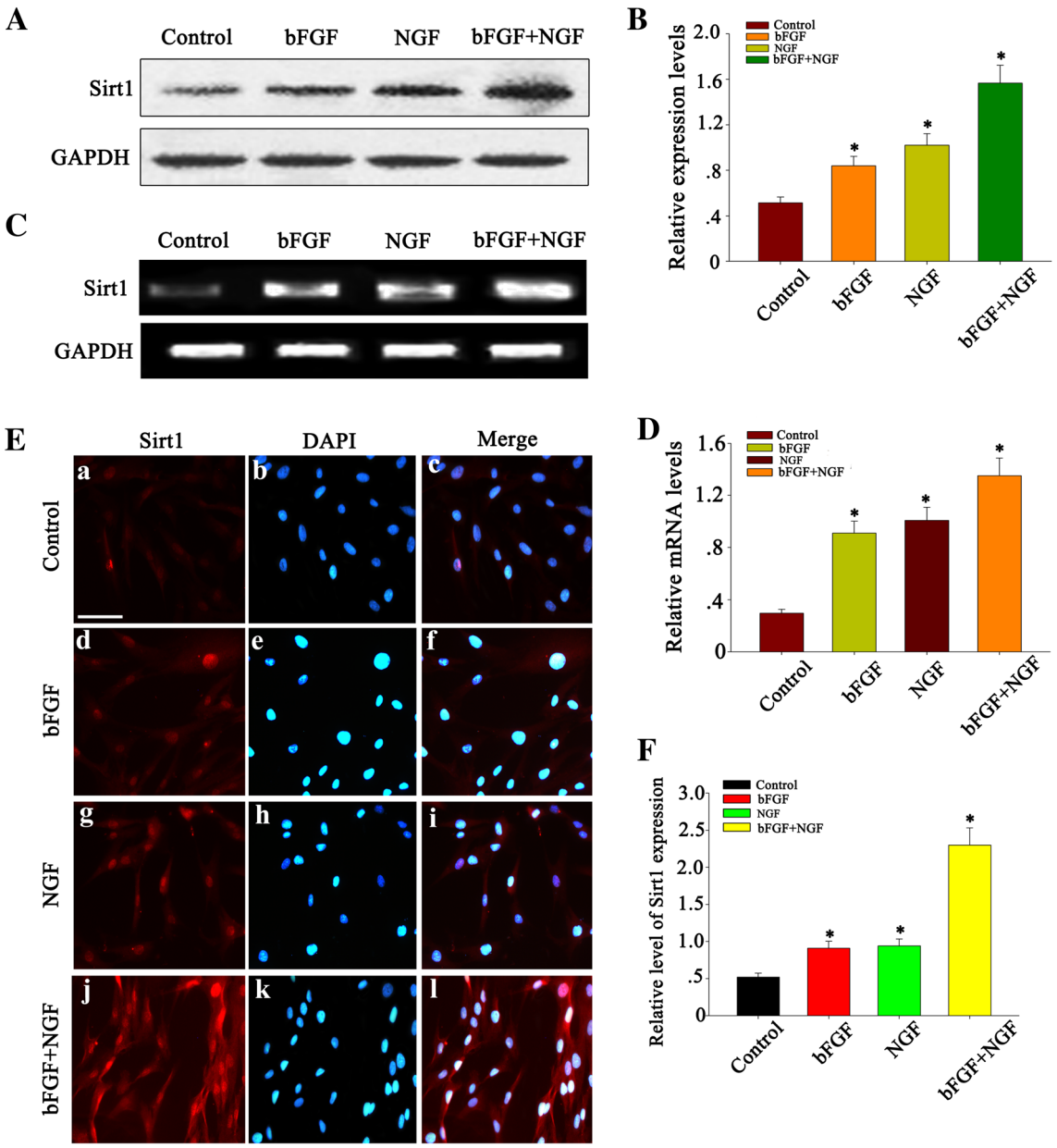

Fig. 2 Expression of Sirt1 increased in DPSCs stimulated with bFGF and NGF during differentiation. **a** After treatment with bFGF and NGF alone or in combination, western blot analysis of Sirt1, cells in neural differentiation medium for 7 days were as control. **b** Quantification of Sirt1 protein levels. $^*P < 0.05$. **c** Total RNA was isolated at 7 days after induction of differentiation, followed by RT-PCR analysis. **d** Quantitation of PCR products. The quantity of amplified product was analyzed by an image analyzer. $^*P < 0.05$. **e** Immunocytochemisry of Sirt1 (*Blue*, DAPI. original magnification: ×200). *Scale bar* = 50 μm. **f** Quantification of Sirt1 positive cells. The Sirt1-positive cell ratio was counted by using phase-contrast microscopy ($^*P < 0.05$). (Color figure online)

examine the protein expression levels of ERK and AKT in the various groups. As shown in Fig. 3, the expression levels of p-AKT and p-ERK were increased in the bFGF and NGF induced groups, and was the highest in co-induced groups. These results indicated that the MAPK pathway may manipulate the neural differentiation of DPSCs. To investigate the effect of Sirt1 on bFGF and NGF induced neural differentiation of DPSCs, we used Sirt1 inhibitor. After treatment of Sirt1 inhibitor, the expression levels of p-AKT and p-ERK were decreased (Fig. 3c, d), suggesting that Sirt1 enhanced the phosphorylation of AKT and ERK. Resveratrol (trans-3,5,4-trihydroxystilbene, RSV), a natural polyphenolic phytoalexin, is highly concentrated in grapes and red wine. There is increasing evidence that resveratrol, a Sirt1 activator, plays a pivotal role in neuroprotection and neuronal differentiation. The RSV-dMSCs showed a higher

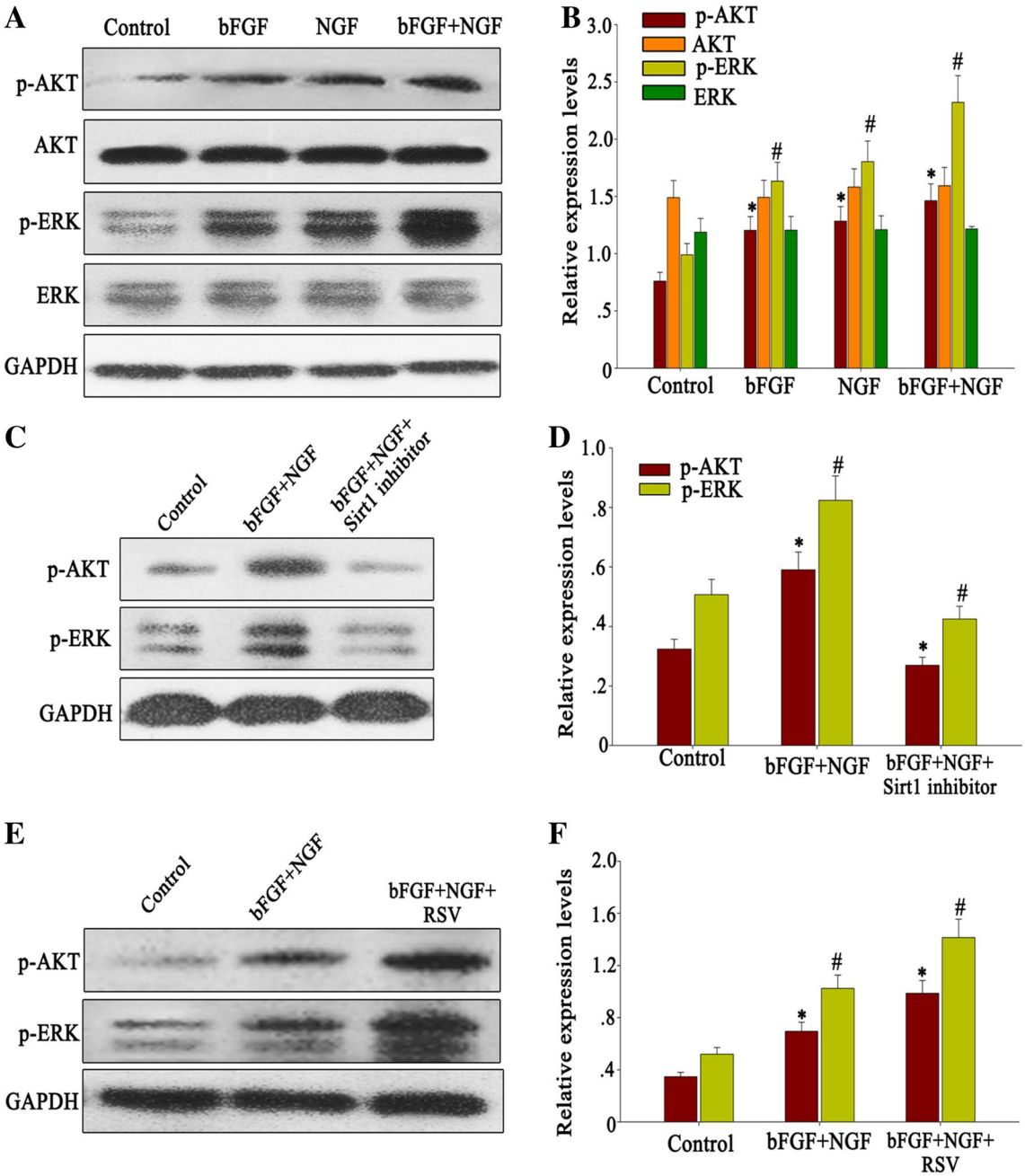

Fig. 3 ERK and AKT signaling were highly active in bFGF and NGF induced DPSCs and reversed by Sirt1 inhibitor. **a** DPSCs were treated with bFGF and NGF alone or in combination, AKT, p-AKT, ERK, and p-ERK expression was determined by Western blot analysis. **b** Quantification of AKT, p-AKT, ERK, and p-ERK protein levels. $^{*}P < 0.05$. **c** DPSCs were cultured in neural differentiation medium containing bFGF and NGF in combination or Sirt1 inhibitor and bFGF, NGF for 7 days. The p-AKT and p-ERK expressions were analyzed by Western blot. **d** Quantification of p-AKT and p-ERK protein levels. $^{*}P < 0.05$. **e** DPSCs were cultured in neural differentiation medium containing bFGF and NGF in combination or Sirt1 activator-RSV and bFGF, NGF for 7 days. The p-AKT and p-ERK expressions were analyzed by Western blot. **f** Quantification of p-AKT and p-ERK protein levels. $^{*}P < 0.05$

expression of the neuronal marker proteins, Nestin and NF-M [27]. Our data showed that after treatment of Sirt1 activator-RSV, the expression levels of p-AKT and p-ERK were increased, suggesting that Sirt1 enhanced the phosphorylation of AKT and ERK (Fig. 3e, f).

Sirt1 Promoted bFGF and NGF Induced Neural Differentiation of DPSCs

In the present study, after treatment of Sirt1 inhibitor, the expression levels of p-AKT and p-ERK were

decreased,western blot analysis showed that Sirt1 promoted the phosphorylation of AKT and ERK, furthermore ERK and AKT signaling were highly active in bFGF and NGF induced DPSCs, then we considered that whether Sirt1could enhance bFGF and NGF induced neural differentiation of DPSCs. Western blotting detected the protein expression levels of Nestin, MAP-2, βIII-tubulin and GFAP in the various groups. Notably, relative protein expression levels were lower in the Sirt1 inhibitor group, as compared with the bFGF and NGF co-induced group (Fig. 4a, b). When levels of neuronal markers mRNA were analyzed by RT-PCR, the increase in neuronal markers protein secretion correlated with the accumulation of neuronal markers mRNA (Fig. 4c, d). After treatment with Sirt1 inhibitor, the immunofluorescence staining showed that the number of positive cells had markedly decreased (Fig. 4e). These observations require that Sirt1 may promote bFGF and NGF induced neural differentiation of DPSCs.

Discussion

Currently, cell therapy for neurological diseases has been analyzed using MSCs derived from bone marrow, adipose

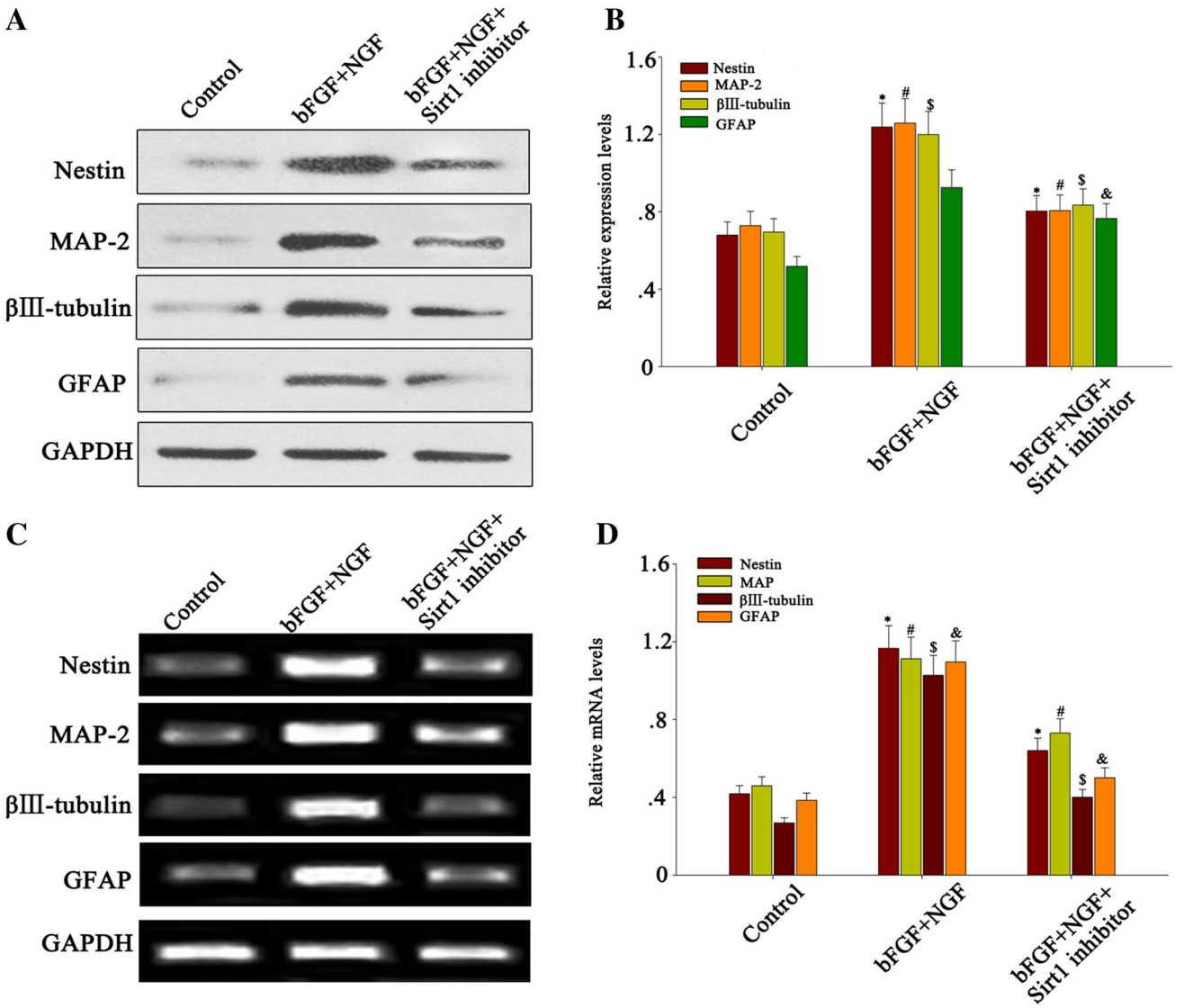

Fig. 4 Sirt1 promoted bFGF and NGF induced neural differentiation of DPSCs. **a** DPSCs were cultured in neural differentiation medium containing bFGF and NGF in combination or Sirt1 inhibitor and bFGF, NGF for 7 days, expressions of Nestin, MAP-2, βIII-tubulin and GFAP were analyzed by Western blot. **b** Quantification of Nestin, MAP-2, βIII-tubulin and GFAP protein levels. $^*P < 0.05$. **c** Total RNA was isolated at 7 days after induction of differentiation, followed by RT-PCR analysis. **d** Quantitation of PCR products. The quantity of amplified product was analyzed by an image analyzer. $^*P < 0.05$. **e** Immunofluorescence staining of Nestin, MAP-2, βIII-tubulin and GFAP (original magnification: ×200). *Scale bar* = 50 μm

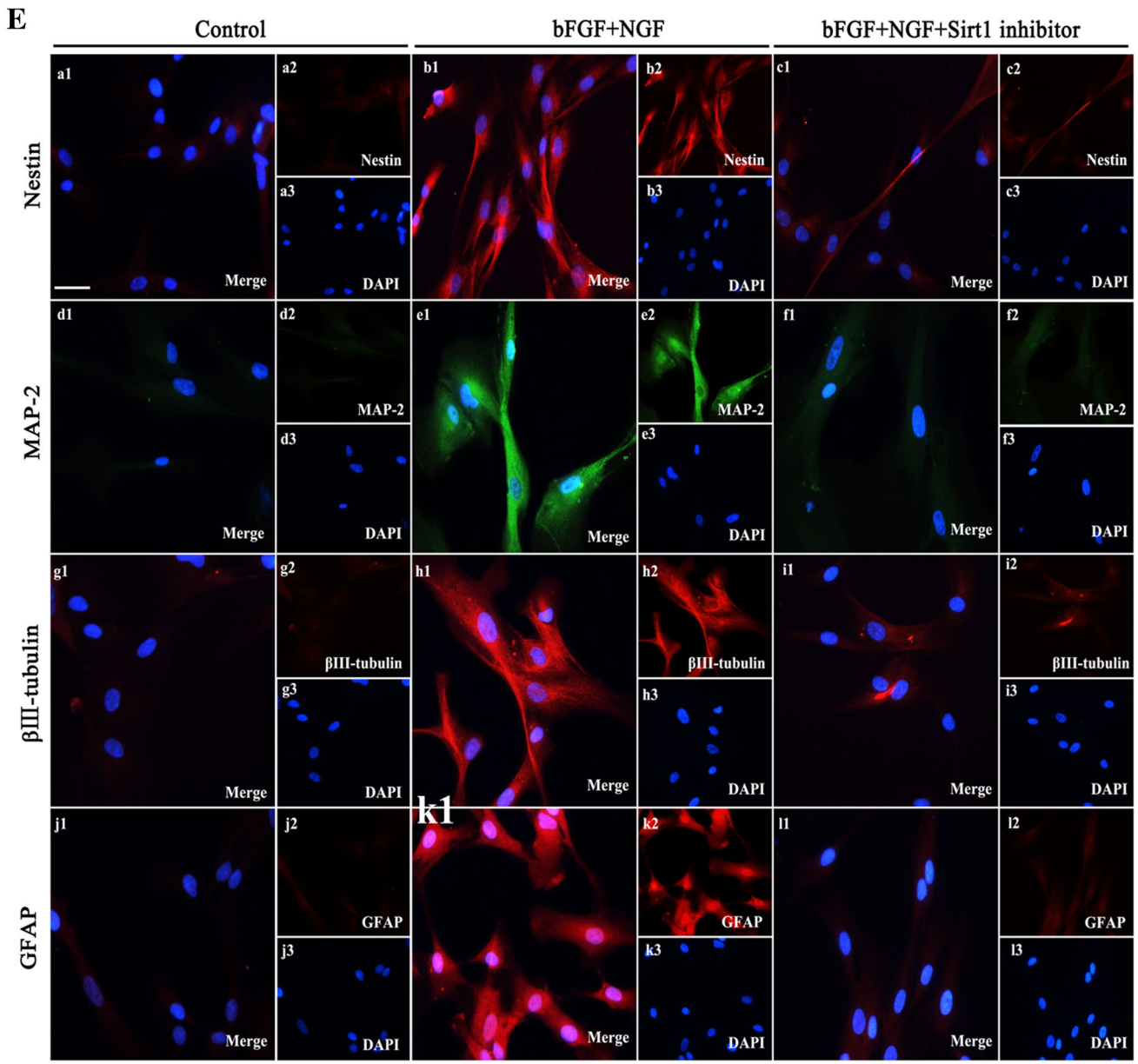

Fig. 4 (continued)

tissue, embryonic stem cells, and neural stem cells [30]. The nervous system consists of two types of cells, neurons and glial cells. The underlying repair mechanisms of cell therapy for neurological diseases are the secretion of neurotrophins from seed cells, and ultimately the differentiation of seed cells into neurons and glial cells. Therefore, the identification of a suitable seed cell that secretes neurotrophins and be easily to be induced the neuronal differentiation is important for the treatment of neurological diseases and for the repair of neuronal damage.

DPSCs have the advantage of convenience sampling, easy expansion, and possess the ability to differentiate into neurons; therefore, DPSCs are regarded as a promising seed cell in tissue engineering for the treatment of neurological diseases [31, 32]. The transplantation of human DPSCs was demonstrated to improve motor capacity in a mouse spinal cord injury model, and stem cells from human exfoliated deciduous teeth promoted locomotor recovery following transection of rat spinal cords [6, 33]. bFGF and NGF are important neurotrophins, which possess superior properties, when compared with other types of neurotrophic factors, in the maintenance of neuronal survival, anti-apoptotic function in neurons, and promotion of MSCs differentiation into neuron-like cells *in vitro*

[7]. In this study, we evaluated the potential of DPSCs to differentiate into multiple types of neural cells. Analysis of differentiated cells by western blot and RT-PCR proved that DPSCs + bFGF and NGF alone or combination could indeed differentiate into Nestin$^+$ neural stem cell, MAP-2$^+$ neurons, βIII-tubulin$^+$ neurons and GFAP$^+$ astrocytes. We identified that the levels of Nestin, MAP-2, βIII-tubulin and GFAP the most highest in the DPSCs + bFGF + NGF group compared with the other groups.

Previous study found that NGF treatment induced Sirt1 gene and protein expression of the PC12 cells in the low glucose DMEM [34]. There is increasing evidence that Sirt1 activation has a key role on neuronal architecture by stimulating axon elongation and neurite outgrowth [23–25]. Previous studies have shown that resveratrol (a Sirt1 activator) treatment, along with the use of neuronal induction media, effectively stimulates neuronal cell differentiation of bone marrow MSCs [27]. Glial cells play pivotal roles in neuronal development, activity and plasticity [35]. It is well known that glial cells are also involved in providing neurotrophic signals to neurons required for their survival, proliferation, and differentiation. GFAP is expressed in the central nervous system in astrocytes. It is involved in many important CNS processes, including cell communication and functioning of the blood–brain barrier [36]. These studies suggest that the Sirt1 activation is critical for inducing neural differentiation of MSCs. In this study, we found Sirt1 protein and mRNA levels in bFGF-treated and NGF-treated groups significantly increased especially in co-treated groups.

As a downstream molecule of Sirt1, AKT can be modulated by Sirt1 through deacetylation [23, 37]. Besides, AKT has multiple roles in regulating neuronal cell size and survival, accelerating axonal regeneration, and promoting axon elongation and branching [38–41]. A recent study revealed that NGF induced the neuritogenesis in dopaminergic cells via two distinct processes, namely, the early ERK-driven and transcription-dependent latency process, and the later ERK- and PI3K/AKT- driven and transcription-independent neurite extension process [42]. Extracellular signal-regulated kinases, a well known members of the MAP kinase family, act as integration points for multiple biochemical signals, and in addition they are involved in a wide variety of cellular processes, such as proliferation, differentiation, transcription regulation, and development [43]. The activation of this kinase requires its phosphorylation by upstream kinases. It has been widely demonstrated that Ras GTP binding proteins are involved in the activation of ERKs [44]. Many different stimuli, including growth factors, cytokines, virus infection, ligands for heterotrimeric G protein-coupled receptors, transforming agents, and carcinogens, activate the ERK pathway [43]. The MAPK/ERK pathway is a well-known chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell. It is hypothesized that the cellular response to extracellular signaling agents, such as hormones and GFs, may induce stimulation or inhibition of specific functions associated with some cellular compartments or with the nucleus. Often, the response is a modification of gene expression [45]. It has been reported exogenous factor induced differentiation of MSCs, suggesting that the ERK pathway is involved in the neural differentiation of MSCs [46, 47]. In addition, it is well known that growth factors are mitogenic polypeptides playing a crucial role during astroglial and neuronal cell proliferation and differentiation in culture [48]. Previous study demonstrated that NGF and bFGF co-transfected MSCs exhibited an increased expression of ERK phosphorylation in MSCs, as well as increased proliferation and neural differentiation [7]. Similar to previous study, DPSCs were incubated with/without 1 μM resveratrol for 12 h. However, pre-induction media of previous study contained DMEM, 10% FBS, 10 ng/mL bFGF, and 500 μM β-mercaptoethanol for 24 h [27]. Our data showed that after treatment of Sirt1 activator-RSV, the expression levels of p-AKT and p-ERK were increased, suggesting that Sirt1 enhanced the phosphorylation of AKT and ERK. The results of the present study demonstrated that bFGF and NGF stimulated together exhibited increased expression levels of p-AKT and p-ERK, whereas the control DPSCs exhibited lower expression of p-AKT and p-ERK, thus suggesting that that ERK and AKT signaling pathway is involved in regulation of DPSCs neural differentiation.

In conclusion, the present study examined the effects of bFGF and NGF on neural differentiation of DPSCs. The results indicated that bFGF and NGF exerted a synergistic regulatory effect on DPSCs neural differentiation. Thus, the present study provides insight into the use of tissue engineering technology for future treatment of neurological diseases and for the repair of neuronal damage.

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