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Original Article

Selegiline induces adipose tissue-derived stem cells into neuron-like cells through MAPK signaling pathway

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ABSTRACT

Introduction: Adipose-derived stem cells (ADSCs) are one of the most well-known and accessible sources of stem cells that can be used for the treatment of neurodegenerative diseases. On the other hand, previous studies have suggested that selegiline, as an irreversible inhibitor of monoamine oxidase, affects stem cells' differentiation into neurons. This study was conducted to investigate the involvement in phosphatidylinositol-bisphosphate 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways in ADSCs differentiation to neuron-like cells using selegiline as inducer.

Methods: ADSCs were isolated from male rats, cultured in DMEM and then treated with selegiline (10^{-7} M) for 24h. Real-time PCR for nestin and neurofilament-68 (NF-68) was performed from the negative control (ADSCs at the 3rd passage), positive control (ADSCs were treated with 10^{-7} M selegeline for 24h, PI3AKT inhibitor (ADSCs were pretreated with treated with 10µM LY294002 for 3h, then10^{-7} M selegeline for the next 24h, and MAPK inhibitor (ADSCs were pretreated with treated with 10µM PD98059 for 3h, then10^{-7} M selegeline for the next 24h).

Results: Nestin and NF-68 genes have been over-expressed in the selegiline-treated ADSCs. The PD98059 and LY294002 significantly down-regulated the selegiline-induced over-expression of nestin and NF-68; however, PI3K inhibition did not return the genes expression to control level. ADSCs were immunoreactivefor nestin and NF-68 about 98% and 95% respectively.

Conclusion: According to the results, selegilinecan induce the gene expression of neural stem cell biomarkers in ADSCs through MAPK pathway activating and so differentiating them into neuron-like cells.

Keywords:
Selegiline
Adipose-derived stem cells
Neuron-like cells
MAPK

Introduction

Central nervous system injuries are among the most harmful lesions in people's life and so their treatment is highly considered (Modrak et al., 2020). Stem cells are suitable for replacing old and damaged cells due to their ability to grow and dif-

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ferentiate to other cells, so are used in treatment as cell therapy (Wei et al., 2017). Adult stem cells (mesenchymal stem cells) are multi-potent undifferentiated cells that are found in many differentiated tissues (Wang, 2019). These cells can trans-differentiation which regardless of producing the same tissue cells, they can generate other tissue cells a phenomenon named plasticity (Dominici et al., 2006). Mesenchymal cells express a wide variety of cell surface markers and lack the markers of hematopoietic cells (Alhadlaq and Mao, 2004). Mesenchymal stem cells, due to the ability of differentiate into different lineages, are the best selection for cell therapy in neurodegenerative diseases. Also, these cells can be isolated from different animal species and different tissues of the body (Fan et al., 2020). Adipose derived stem cells (ADSCs) are one of the types of adult stem cells (Mizuno, 2009). The adipose tissue sample contains mature adipocytes and adipose tissue stromal cells composed of immature fat cells, smooth muscle cells, fibroblastic cells, endothelial cells, immune cells and blood cells (Fraser et al., 2006). Adipose tissue stromal cells have features such as self-renewal capacity, pluripotent ability fibroblast-like morphology, ability of attachment to culture dish and the potential of differentiation to other mesenchymal lines (Sylvester and Longaker, 2004). A simple incision in the skin or fat tissue during liposuction can be used to prepare of ADSCs samples and there is no age limit for fat tissue. ADSCs are able to differentiate into mesenchymal lineages, in addition they can differentiate into ectodermal lineage such as neural and glial cells by neural inducer (Huang et al., 2007). Stem cells are considered as a suitable source of cell therapy (Alhadlaq and Mao, 2004). ADSCs can be used as the first option due to their easy and painless access, neither age limit nor phenotypic changes after several passages (Hong et al., 2006). According to previous studies, the amount of stem cells in the adipose tissue is 500 times higher than the bone marrow sample and attachment time course of ADSCs to a culture dish is less than 12h. ADSCs could express neurotrophic factors in *in vivo* condition (Hong et al., 2006; Huang et al., 2007). It has been observed that after injection of ADSCs into ischemic brain, these cells could differentiate into neuronal cells that can secrete growth factors and cytokines (Cardozo et al., 2012). Neural inducers are among the most important factors in cellular differentiation (Bai et al., 2020). Previous studies have suggested that selegiline, as irreversible inhibitor of monoamine oxidase, is effective in differentiating stem cells into neuron like cells by altering the expression of some of the genes and proteins in the cell (Esmaeili et al., 2006). Selegiline was initially used as an antidepressant drug, but later determining its inducer role, it was used to treat neurodegenerative disorders such as Parkinson's disease (Mizuta et al., 2000). It causes proliferation of the neural and neuroglial cells through neurotrophins stimulation. Mizuta et al. (2000) have shown that selegiline increases nerve factors such as nerve growth factor (NGF), glial cell-derived neurotrophic factor and brain-derived neurotrophic factor (BDNF). As a neuroprotective drug, it can induce bone marrow stromal cells and differentiate them into neuron-like and glial-like cells *in vitro* (Abdanipour et al., 2018). Nerve cell biomarkers are used to prove the nature of a neural cell in which nestin and neurofilament-68 (NF-68), an intermediate filament in the neurons is well characterized (Boulland et al., 2013). According to previous studies, most of the growth factors and other stimuli work through mitogen-activated protein kinase (MAPK) and phosphatidylinositol-bisphosphate 3-kinase (PI3K)/AKT signaling pathways to regulate neuronal survival (Pearson et al., 2001; Wang et al., 2016). Initiating these pathways has been implicated in various physiological functions including differentiation, proliferation, metabolism, migration, cell death and life (Dinsmore and Soriano, 2018). Recent studies have shown that treatment of ADSCs with selegiline induces their differentiation into neuron-like cells (Abdanipour et al., 2011; Abdanipour and Tiraihi, 2012). Furthermore, a couple of studies have shown that selegiline and its newer derivative rasagiline could affect MAPK signaling pathway (Am et al., 2004; Andoh et al., 2005; Naoi et al., 2020). However, the exact mechanism of selegiline effect in promoting ADSCs into neuron-like cells is unknown. To find out the intracellular mechanism of this phenomenon, we aimed to investigate nestin and NF-68 gene expressions potential selegiline

targets, and study the involvement of PI3K and MAPK pathways in this effect of selegiline.

Material and methods

Preparations of ADSCs

All the experiments were carried out under the ethical guidelines of Zanjan University of Medical Sciences (IR.ZUMS.REC.1397.50). ADSCs were collected from the perinephric and para-epididymal fat tissues from adult Wistar rats. Harvested ADSCs were washed several times in the sterile phosphate buffer solution (PBS, containing 100U/ml penicillin, 100µg streptomycin) and then incubated for 30min at 37ºC in 0.075% phosphate buffer type-1 collagenase (sigma) with shaking. Attained suspension was filtered with 100µm nylon filter (falcon company), neutralized with 10% fetal bovine serum and then centrifuged (1000 rpm, 10min). The supernatant was discarded and collected ADSCs were cultured in DMED containing 10% fetal bovine serum (Chemi-Con), penicillin/streptomycin (100U/ml penicillin, 100µg streptomycin) and then incubated at 37°C. ADSCs were identified using CD90 (mesenchymal stem cells marker) and ADSCs specific marker CD49d. Osteogenic differentiation and Alizarin Red S staining were used for evaluating multipotential properties of the ADSCs. Cells were cultured in the osteogenic medium containing 10mM β-glycerophosphate, 0.2mM ascorbic acid and 10^{-7}M dexamethasone for 21 days.

Neural induction and design experimental study

ADSCs (3rdpassage) were assigned to the different experimental groups as follows: the negative control, positive control (10^{-7} M selegeline for 24h), PI3AKT inhibitor (ADSCs were pretreated with treated with 10µM LY294002 for 3h, then10^{-7} M selegeline for the next 24h) and MAPK inhibitor (ADSCs were pretreated with treated with 10µM PD98059 for 3h, then 10^{-7} M selegeline for the next 24h).

Immunocytochemistry

In the positive control group, the percentage of immunoreactive cells to nestin and NF-68 was used to evaluate the neural induction. The isolated and induced ADSCs were plated on a gelatin-coated glass coverslip, washed in PBS and fixed with 4% paraformaldehyde in PBS for 15min. The fixed cells were washed twice with PBS before staining. The cells were treated with the blocking buffer (0.1% Triton X-100, 10% goat serum in PBS) for one hour (Triton x-100 used for cytoplasmic antigens). The primary antibodies: mouse anti- CD 49d, polyclonal antibody (1:300; Millipore), mouse anti-nestin polyclonal antibody (1:100; Millipore) and mouse anti-NF-68 monoclonal antibody (1:50; Millipore) were incubated overnight at 4°C. Then, the cells were washed three times with PBS, incubated with secondary antibody (anti-mouse FITC-conjugated and anti-rabbit FITC-conjugated; 1:100, both from Millipore) for two hours at room temperature. The cells were washed with PBS twice and counterstained with ethidium bromide for one min to demonstrate the nuclei. Finally, they were washed in PBS and examined at 200×magnifications (Olympus).

Real-time RT-PCR (RT-PCR)

The RT-PCR was carried out using of 1000ng purified RNA obtained by TRIzol (Invitrogen; Thermo Fisher Scientific, Inc). Revert Aid™ First Strand cDNA Synthesis Kit (Fermentas, Germany) was used to synthesize 20µl cDNA. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control for normalization. RT-PCR was conducted using primers shown in Table 1. Each PCR reaction was performed in a 12.5µl final reaction volume for 40 cycles at 95°C for 15s followed by 60°C for 1min. For analyzing relative changes in mRNA levels, we used delta $C_{T\ method.}$

Statistical analysis

Statistical analyses were performed using SPSS software version 15 (IBM; Armonk, New York, United States). All data are presented as mean±SEM from 5 independent experiments. The one-way ANOVA followed by Tukey's post hoc was used to make a statistical comparison between groups. The level of significance was set at $P<0.05$.

Results

Preparations of ADSCs

The primary culture of the isolated ADSCs showed

TABLE 1: Primer sequences and PCR parameters. Primers for amplification of target sequences, their Gen Bank accession number and size of the fragment amplified are presented.

Gene	Gene Accession no.	Sense 5 → 3	Anti-sense 5 → 3	bp
Nestin	NM_001308239	CAAATCTGGGAACTGGTAGAG	CCTAGAGCCTTCAGTGTTTC	149
Nf-68	NM_031783	ATATGCAGAATGCCGAAGAG	CTTCGATCTCCAGGGTCTTA	147
GAPDH	NM_017008	GCCTCCAAGGAGTAAGAAAC	GTCTGGGATGGAATTGTGAG	141

Primers were designed by Gene Runner 3.05 software (Product by: info@genfanavaran.com). *Nestin*, a cytoskeletal intermediate filament initially characterized in neural stem cells; *Nf-68*, neurofilament light (Nefl); *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase.

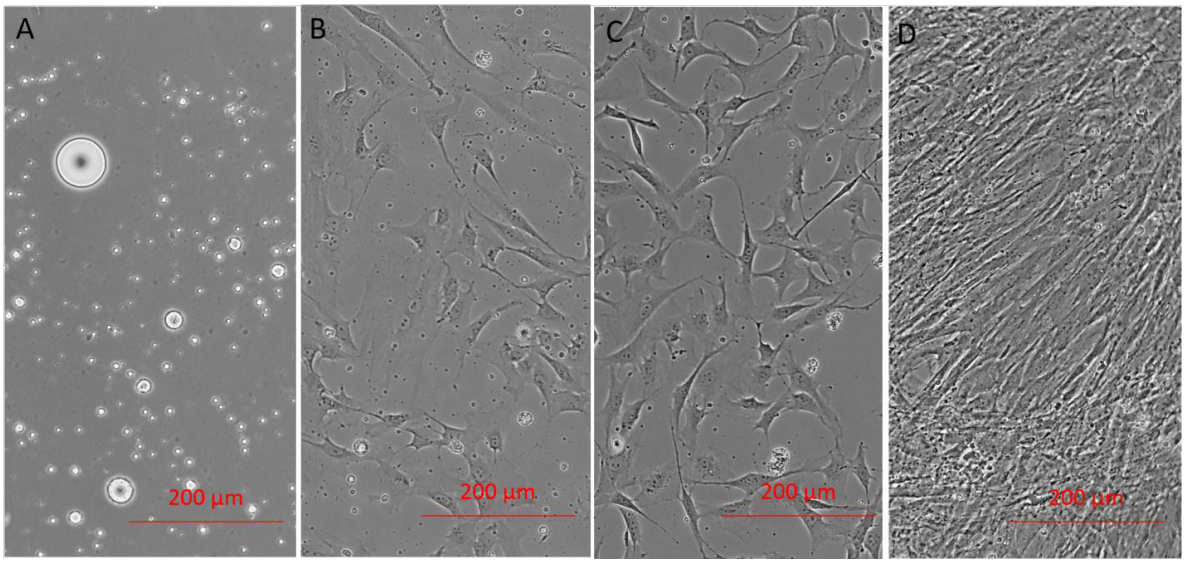

FIGURE 1. Representative photomicrographs of adipose derived stem cells (ADSCs) isolation and culture. A: represents floating cells at 2 days; B: attached fibroblast-like cells (day of 7); C and D: represents first and third passages at days 7 and 22, respectively. Scale bar: 200µm.

that ADSCs adherent to plastic flask similar to mesenchymal stem cells and was characterized by a rapid proliferation (Figures 1A-C). At earlier hours, the cells were floating and their nucleus visible (Figure 1A). After 24h, the floated cells were attached to dish to form fibroblast-like colonies. The first passage was made after 9 days when the cells reached 75% confluency (Figure 1C). After the first passage, they exhibited extensive proliferative capacity. The second passage was performed in the following 7 next days (Figure 1C). Third passage cells were used as indicated for all experiments (Figure 1D), then were immunostained with anti- CD49d (specific marker for fat cells, Figures. 2A-D) and CD90 (Figures. 2E-H). Results showed that the percentage of immunoreactive cells to CD90 and CD49d were 100%. The *in vitro* differentiation of ADSCs into osteogenic phenotypes using induction cocktail medium has been shown in Figure 2Q. The results showed that osteoblast-like cells were capable of mineralizing extracellular matrix and staining with Alizarin Red S dye.

Neural induction

ADSCs were cultured in 10^{-7} M selegeline for 24h resulting cells were positive for nestin (Figures 2C-F) and NF-68 (Figures 2B-E) immunoreactive (98% and 95%; respectively) as compared with negative control group (ADSCs at the 3rd passage without any treatment). In the time course study of neural phenotype induction, incubation with selegiline resulted in retraction of cell body and processes and these changes were noticed during 2h.

Neuronal phenotype

In order to investigating the effect of selegiline on cell morphology, ADSCs were cultured with 10^{-7} M concen-

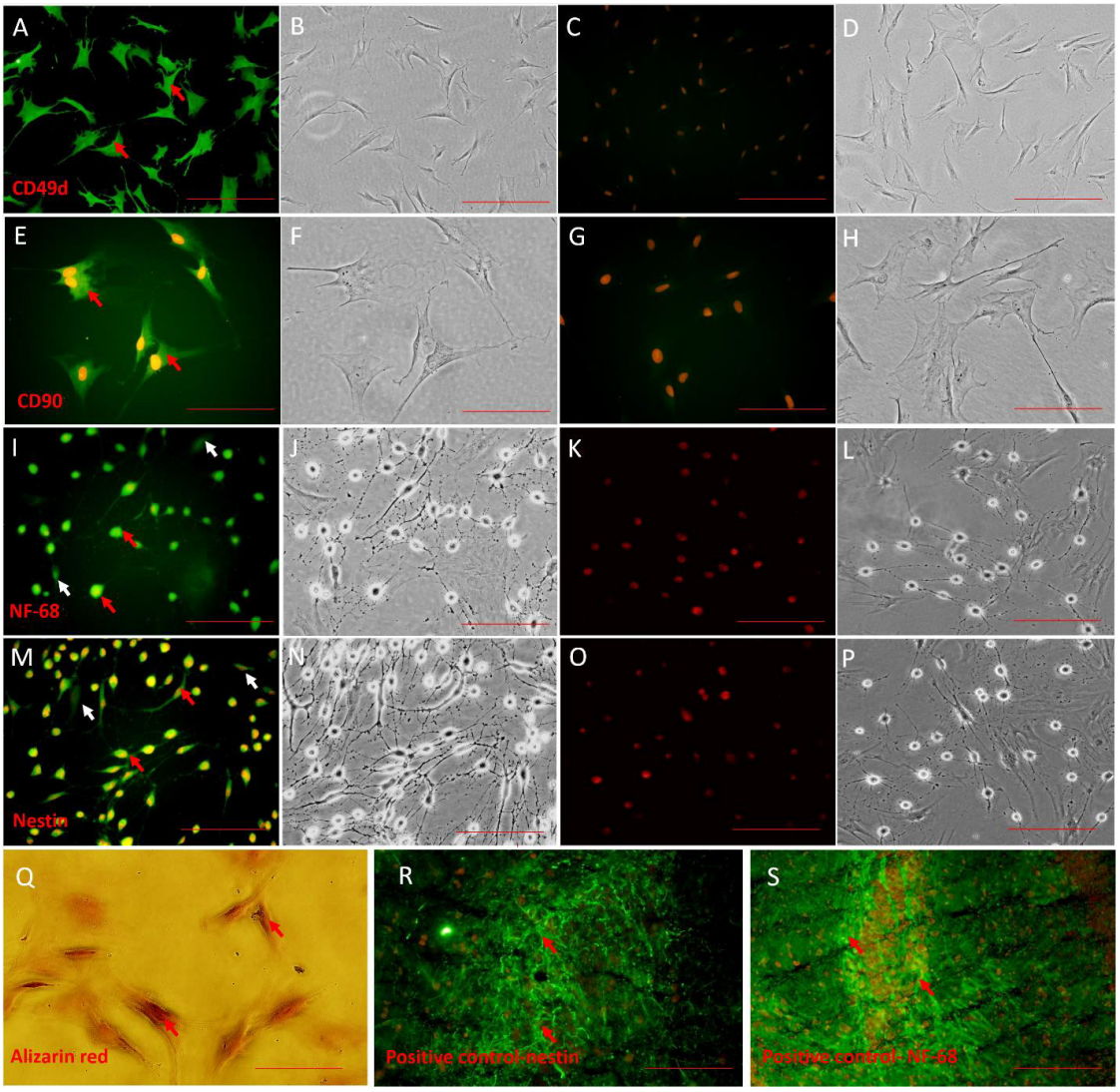

FIGURE 2. Representative photomicrographs of adipose derived stem cells (ADSCs) and induces cells. A, E, I and M: represents immunostaining of CD49d (ADSCs specific marker), CD90 (mesenchymal stromal cell marker), NF-68 (neuron like cells marker) and nestin (marker for neural stem cells progenitor cells); respectively. B, F, J and N: represents phase contras at the same field. C, G, K and O: represent negative control. D, H, L and P: represents phase contras at the same field. Q, R and S: represent alizarin red staining, positive nestin and NF-68 antibodies in the neonatal rat brain, respectively. The cells were immunostained with relevant primary antibody and labeled with FITC-conjugated secondary antibody (green color shows positive cells) and the red colors are ethidium bromide counterstaining of the nuclei. Scale bar: 200μm.

tration of selegeline for 24h (without and in presence of inhibitors). Results showed that there was shrinkage in cell-body of induced ADSCs' and they exhibited neuritis with the neuronal phenotype appear. As well, the use of PI3AKT and MAPK inhibitors resulting in a significant decrease of the neuronal phenotype, especially when using MAPK inhibitor. The results are presented in the Figures 3A-D.

Gene expression results

The results of RT-PCR were shown in the Figure 4. The results demonstrated a significant decreases of mRNA levels of *nestin* and *NF-68* genes in PI3AKT and MAPK inhibitors groups (*nestin*: 1.54±0.18 and 0.32±0.08; *NF-68*: 2.45±0.26 and 0.58±0.02; respectively) in comparison with positive control (*nestin*: 4.95±0.46; *NF-68*: 3.94±0.39) group ($P<0.05$). On the other hand, PI3K inhibition by LY294002 did not return the genes expression to control level.

Discussion

During this study, adipose tissue cells were easily and in great amount harvested. These cells were het-

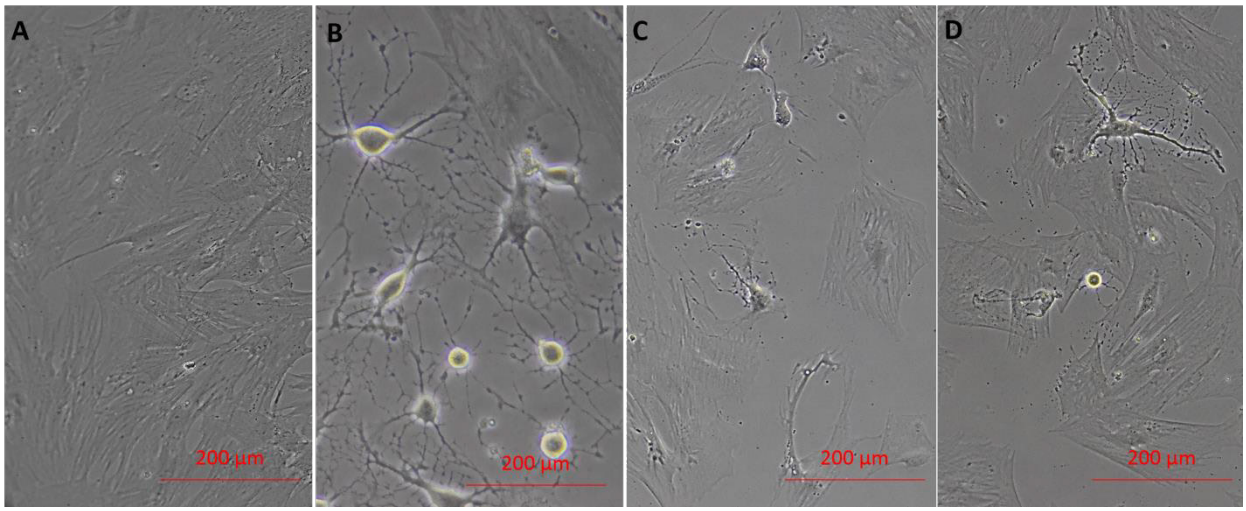

FIGURE 3. Phase contrast images of cell morphology. A: Adipose derived stem cells (ADSCs) treated with 0M selegiline as a negative control; B: ADSCs treated with 10^{-7}M concentration of selegiline; C: ADSCs pretreated with PI3AKT inhibitor then 10^{-7} M selegiline; D: ADSCs pretreated with MAPK inhibitor then 10^{-7} M selegiline. Scale bar: 200µm.

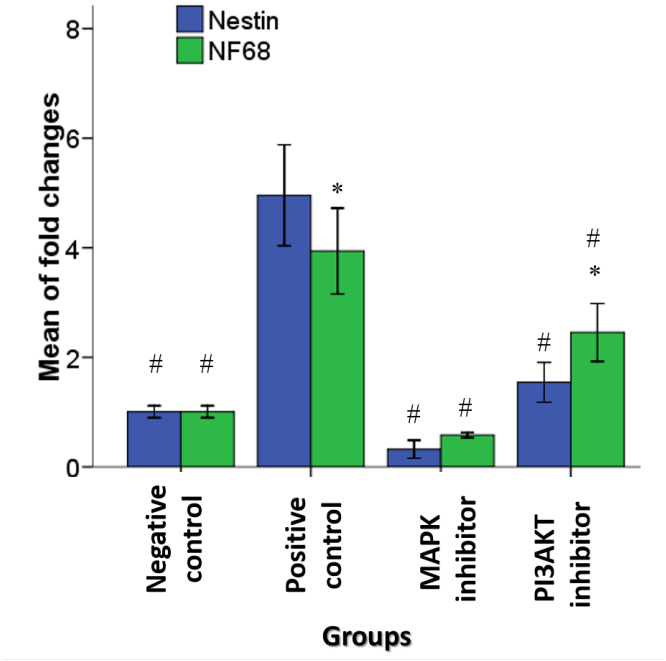

FIGURE 4. Quantitative real-time RT-PCR results, relative to non-treated adipose derived stem cells (ADSCs) (0 µM concentration of selegiline), normalized to GAPDH mRNA amplification. Represents nestin and NF-68 mRNA levels for the negative control (ADSCs at the 3rd passage), positive control (ADSCs were treated with 10^{-7} M selegeline for 24h), PI3AKT inhibitor (ADSCs were pretreated with treated with 10µM LY294002 for 3h, then 10^{-7} M selegeline for the next 24h) and MAPK inhibitor (ADSCs were pretreated with treated with 10µM PD98059 for 3h, then10-7 M selegeline for the next 24h). The bars indicate the mean±SEM; $^{*}P<0.05$ compared with negative control, $^{\#}P<0.05$ compared with positive control.

erogeneous at the beginning of culture and contain a small number of endothelial cells, smooth muscle and pericytes. The number of these cells was reduced by increasing the passages and was considered to be purified. This finding was consistent with the results of previous studies (Fraser et al., 2006). The ADSC identity was confirmed by CD49d and CD90 (ADSC and/or MSC specific markers) that is consents with other research (Debnath and Chelluri, 2019).

Selegiline, as an irreversible inhibitor of MAO-B, has anti-apoptotic and neuroprotective effects (Magyar and Szende, 2004). This drug, by altering the genes and proteins expression in the cell, causes stem cells differentiation into neuronal and glial cells (Kingham et al., 2007). Taheri et al. showed that selegiline, compared with other inducers, increased cell survival and proliferation and could be used as a cell inducer (Taheri et al., 2012).

Our results showed that ADSCs induced by selegiline, was able to express nestin and NF-68 genes and protein production. Abdanipouret et al. (2011) have report-

ed that neuron-like cells produced in the pre-induction stage could express the nestin, NF-68 and neurotrophins (Abdanipour et al., 2011). Esmaeili et al. (2006) has also shown that selegiline differentiates embryonic stem cells into neuron-like cells and induced cells can express neurotrophins. According to Mizuta et al. (2000) selegiline increased the gene expression of neurotrophic factors of BDNF, NGF and neurotrophin-3 in mouse astrocytes in a culture medium (Mizuta et al., 2000). Several studies have been conducted on the growth and differentiation inducers' signaling pathways in the stem cells. MAPKs are a group of protein-serine/threonine kinases, which participate in signal transduction pathways that control intracellular events including responses to hormones and developmental changes in organisms (Pearson et al., 2001). Also, several neurotrophic growth factors, such as BDNF, trigger the PI3K/ AKT signaling pathways and through activating tyrosine kinase receptor, increase neuronal survival and differentiation (Mizuta et al., 2000) .

To evaluate the intracellular mechanism of selegiline in our induced cells we investigated the involvement of some signaling pathways. As shown previously by Andoh et al. (2005) selegiline could activate MAPK/ Erk1/2 downstream to protein kinase A. The same has been reported by other groups regards to rasagiline, another derivative of selegiline (Am et al., 2004; Weinreb et al., 2006). Based on the results of this study, selegiline modulated nestin and neurofilament genes expression in ADSCs and MAPK inhibition by PD98059 removed the up-regulating effect of selegilineon the mentioned genes' expression. So, it is supposed that this effect of selegiline on ADSCs could be occurred via the MAPK. As shown previously by Yuan et. al. (2013), NGF gene induction could increase the differentiation of the bone marrow mesenchymal stem cells (BMSCs) into the neurons by regulating the AKT and the MAPK signaling pathway. Similar to our study, it can be said that MAPK is involved in regulating the differentiation of the mesenchymal cells to neuron like cells. A couple of studies have shown that BMSC's could prevent neuronal apoptosis by producing protective factors. BMSC also increases the survival rate of astrocytes and it is notable that these effects were performed by stimulating PI3K/ AKT and MAPK/ ERK1,2 signaling pathways (Isele et al., 2007). Furthermore, Shan et al. (2008) revealed that retinoic acid is involved in the differentiation of mouse neuronal precursor cells by increasing the expression of active c-Jun N-terminal kinase (JNK). JNKs, as a part of the MAPK family, play a role in the stress signaling, differentiation and neuron cells morphology (Amura et al., 2005) .

We also demonstrated that inhibiting PI3K by LY294002 (10μM) could slightly reduce the inducing effect of selegiline on ADSCs, indicating that other pathways may be involved in nestin and neurofilament modulation by selegiline. Creson et al. (2009) declared that valproic acid through the ERK and PI3K pathways, causes Bcl2 gene transcription, increase the level of Bcl2 protein and consequently increases cell survival and decreases apoptosis. According to Nakaso et al. (2006), selegiline induces the phosphorylation of Trk-B receptor and then induces PI3K activation. PI3K induces nuclear translocation of Nrf2 and up-regulation of anti-oxidative proteins and increase cytoprotective mechanisms. It seems that, depending on the type of inducer and used cells, the results will be different.

In addition, when PD98059 and LY294002 were used together, no synergic effect was observed and LY294002 altered the inhibitory effect of PD98059 on nestin and neurofilament genes expression. This may indicate crosstalk between MAPK and PI3K pathways. According to a couple of studies, in some situations, there are conflicting behaviors in these pathways' activity (Huang et al., 2013; Moelling et al., 2002).

Conclusion

Overall, we can conclude that selegiline increases the expression of nestin and NF-68 in ADSCs; hence, differentiate them into neuron-like cells through MAPK pathway which requires more studies. It seems that selegiline can be a candidate for treatment of neurodegenerative diseases and activating MAPK pathway could be the main point for increasing effectiveness of ADSC-based cell therapies in the future.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Abdanipour A, Jafari Anarkooli I, Shokri S, Ghorbanlou M, Bayati V, Nejatbakhsh R. Neuroprotective effects of selegiline on rat neural stem cells treated with hydrogen peroxide. Biomed Rep 2018; 8: 41-6. https://doi.org/10.3892/br.2017.1023

Abdanipour A, Tiraihi T, Delshad A. Trans-differentiation of the adipose tissue-derived stem cells into neuron-like cells expressing neurotrophins by selegiline. Iran Biomed J 2011; 15: 113.

Abdanipour A, Tiraihi T. Induction of adipose-derived stem cell into motoneuron-like cells using selegiline as preinducer. Brain Res 2012; 1440: 23-33. https://doi.org/10.1016/j.brainres.2011.12.051

Alhadlaq A, Mao JJ. Mesenchymal stem cells: isolation and therapeutics. Stem Cells Dev 2004; 13: 436-48. https://doi.org/10.1089/scd.2004.13.436

Am OB, Amit T, Youdim M B. Contrasting neuroprotective and neurotoxic actions of respective metabolites of anti-Parkinson drugs rasagiline and selegiline. Neurosci Lett 2004; 355: 169-72. https://doi.org/10.1016/j.neulet.2003.10.067

Amura CR, Marek L, Winn RA, Heasley LE. Inhibited neurogenesis in JNK1-deficient embryonic stem cells. Mol Cell Biol 2005; 25: 10791-802. https://doi.org/10.1128/MCB.25.24.10791-10802.2005

Andoh T, Chock PB, Murphy DL, Chiueh C. Role of the redox protein thioredoxin in cytoprotective mechanism evoked by (-)-deprenyl. Mol Pharmacol 2005; 68: 1408-14. https://doi.org/10.1124/mol.105.012302

Bai WF, Zhang Y, Xu W, Li W, Li M, Yuan F, et al. Isolation and characterization of neural progenitor cells from bone marrow in cell replacement therapy of brain injury. Front Cell Neurosci 2020; 14: 49. https://doi.org/10.3389/fncel.2020.00049

Boulland JL, Mastrangelopoulou M, Boquest AC, Jakobsen R, Noer A, Glover JC, et al. Epigenetic regulation of nestin expression during neurogenic differentiation of adipose tissue stem cells. Stem Cells Dev 2013; 22: 1042-52. https://doi.org/10.1089/scd.2012.0560

Cardozo AJ, Gomez DE, Argibay PF. Neurogenic differentiation of human adipose-derived stem cells: relevance of different signaling molecules, transcription factors, and key marker genes. Gene 2012; 511: 427-36. https://doi.org/10.1016/j.gene.2012.09.038

Creson TK, Yuan P, Manji HK, Chen G. Evidence for involvement of ERK, PI3K, and RSK in induction of Bcl-2 by valproate. J Mol Neurosci 2009; 37: 123-34. https://doi.org/10.1007/s12031-008-9122-2

Debnath T, Chelluri LK. Standardization and quality assessment for clinical grade mesenchymal stem cells from human adipose tissue. Hematol Transfus Cell Ther 2019; 41: 7-16. https://doi.org/10.1016/j.htct.2018.05.001

Dinsmore CJ, Soriano P. MAPK and PI3K signaling: at the crossroads of neural crest development. Dev Biol 2018; 444: S79-97. https://doi.org/10.1016/j.ydbio.2018.02.003

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006; 8: 315-7. https://doi.org/10.1080/14653240600855905

Esmaeili F, Tiraihi T, Movahedin M, Mowla SJ. Selegiline induces neuronal phenotype and neurotrophins expression in embryonic stem cells. Rejuvenation Res 2006; 9: 475-84. https://doi.org/10.1089/rej.2006.9.475

Fan XL, Zhang Y, Li X, Fu QL. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cell Mol Life Sci 2020; 77: 2771-94. https://doi.org/10.1007/s00018-020-03454-6

Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol 2006; 24: 150-4. https://doi.org/10.1016/j.tibtech.2006.01.010

Hong L, Peptan IA, Colpan A, Daw JL. Adipose tissue engineering by human adipose-derived stromal cells. Cells Tissues Organs 2006; 183: 133-40. https://doi.org/10.1159/000095987

Huang T, He D, Kleiner G, Kuluz J. Neuron-like differentiation of adipose-derived stem cells from infant piglets in vitro. J Spinal Cord Med 2007; 30: S35-40. https://doi.org/10.1080/10790268.2007.11753967

Huang W, Zhao Y, Zhu X, Cai Z, Wang S, Yao S, et al. Fluoxetine upregulates phosphorylated-AKT and phosphorylated-ERK1/2 proteins in neural stem cells: evidence for a crosstalk between AKT and ERK1/2 pathways. J Mol Neurosci 2013; 49: 244-9. https://doi.org/10.1007/s12031-012-9822-5

Isele NB, Lee HS, Landshamer S, Straube A, Padovan CS, Plesnila N, et al. Bone marrow stromal cells mediate protection through stimulation of PI3-K/Akt and MAPK signaling in neurons. Neurochem Int 2007; 50: 243-50. https://doi.org/10.1016/j.neuint.2006.08.007

Kingham PJ, Kalbermatten DF, Mahay D, Armstrong SJ, Wiberg M, Terenghi G. Adipose-derived stem cells differ-

entiate into a Schwann cell phenotype and promote neurite outgrowth in vitro. Exp Neurol 2007; 207: 267-74. https://doi.org/10.1016/j.expneurol.2007.06.029

Magyar K, Szende B. (-)-Deprenyl, a selective MAO-B inhibitor, with apoptotic and anti-apoptotic properties. Neurotoxicology 2004; 25: 233-42. https://doi.org/10.1016/S0161-813X(03)00102-5

Mizuno H. Adipose-derived stem cells for tissue repair and regeneration: ten years of research and a literature review. J Nippon Med Sch 2009; 76: 56-66. https://doi.org/10.1272/jnms.76.56

Mizuta I, Ohta M, Ohta K, Nishimura M, Mizuta E, Hayashi K, et al. Selegiline and desmethylselegiline stimulate NGF, BDNF, and GDNF synthesis in cultured mouse astrocytes. Biochem Biophys Res Commun 2000; 279: 751-5. https://doi.org/10.1006/bbrc.2000.4037

Modrak M, Talukder M A H, Gurgenashvili K, Noble M, Elfar J C. Peripheral nerve injury and myelination: Potential therapeutic strategies. J Neurosci Res 2020; 98: 780-95. https://doi.org/10.1002/jnr.24538

Moelling K, Schad K, Bosse M, Zimmermann S, Schweneker M. Regulation of Raf-Akt Cross-talk. J Biol Chem 2002; 277: 31099-106. https://doi.org/10.1074/jbc.M111974200

Nakaso K, Nakamura C, Sato H, Imamura K, Takeshima T, Nakashima K. Novel cytoprotective mechanism of anti-parkinsonian drug deprenyl: PI3K and Nrf2-derived induction of antioxidative proteins. Biochem Biophys Res Commun 2006; 339: 915-22. https://doi.org/10.1016/j.bbrc.2005.11.095

Naoi M, Maruyama W, Shamoto-Nagai M. Rasagiline and selegiline modulate mitochondrial homeostasis, intervene apoptosis system and mitigate α-synuclein cytotoxicity in disease-modifying therapy for Parkinson's disease. J Neural Transm (Vienna). 2020; 127: 131-47. https://doi.org/10.1007/s00702-020-02150-w

Pearson G, Robinson F, Beers Gibson T, Xu B E, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001; 22: 153-83. https://doi.org/10.1210/edrv.22.2.0428

Shan Z Y, Shen J L, Li Q M, Wang Y, Huang X Y, Guo T Y, et al. pCREB is involved in neural induction of mouse embryonic stem cells by RA. Anat Rec (Hoboken) 2008; 291: 519-26. https://doi.org/10.1002/ar.20686

Sylvester K G, Longaker M T. Stem cells: review and update. Arch Surg 2004; 139: 93-9. https://doi.org/10.1001/archsurg.139.1.93

Taheri F, Kashani M, Ghorbanian M, Hosseinpour L. Inductive effect of deprenyl and dimethyl sulfoxide on proliferation and survival of the mesenchymal stem cells. J Gorgan Univ Med Sci. 2012; 14: 10-18.

Wang J, Chen Y, Yang Y, Xiao X, Chen S, Zhang C, et al. Endothelial progenitor cells and neural progenitor cells synergistically protect cerebral endothelial cells from Hypoxia/reoxygenation-induced injury via activating the PI3K/Akt pathway. Mol Brain 2016; 9: 12. https://doi.org/10.1186/s13041-016-0193-7

Wang X. Stem cells in tissues, organoids, and cancers. Cell Mol Life Sci 2019; 76: 4043-70. https://doi.org/10.1007/s00018-019-03199-x

Wei L, Wei Z Z, Jiang M Q, Mohamad O, Yu S P. Stem cell transplantation therapy for multifaceted therapeutic benefits after stroke. Prog Neurobiol 2017; 157: 49-78. https://doi.org/10.1016/j.pneurobio.2017.03.003

Weinreb O, Amit T, Bar-Am O, Sagi Y, Mandel S, Youdim M. Involvement of multiple survival signal transduction pathways in the neuroprotective, neurorescue and APP processing activity of rasagiline and its propargyl moiety. in, Parkinson's Disease and Related Disorders (Springer). 2006; 70: 457-65 https://doi.org/10.1007/978-3-211-45295-0_69

Yuan J, Huang G, Xiao Z, Lin L, Han T J M, biochemistry c. Overexpression of β-NGF promotes differentiation of bone marrow mesenchymal stem cells into neurons through regulation of AKT and MAPK pathway. Mol Cell Biochem 2013; 383: 201-11. https://doi.org/10.1007/s11010-013-1768-6