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Selegiline Differentiates Adult Stem Cells toward Dopaminergic-Like Neurons: A Comparison between Two Cellular Niches of Hippocampal Neurogenesis

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Abstract

Objective: Neural stem cells (NSCs) are suitable therapeutic candidates. Here, we compare the proliferation rate, differentiation potential, and expression levels of specific markers in two groups of cultured NSCs derived from rat subgranular (SGZ) and subventricular (SVZ) zones.

Materials and Methods: In this experimental study, NSCs isolated from SGZ and SVZ were cultured in α-minimal essential medium (α-MEM) supplemented with 1% penicillin/streptomycin, 10% fetal bovine serum (FBS), 20 ng/ml basic fibroblast growth factor (bFGF), 20 ng/ml epidermal growth factor (EGF), and B27 supplement. Glial fibrillary acidic protein (*Gfap*), p75 neurotrophin receptor (*Ngfr*), tyrosine kinase receptor A (*TrkA*), beta-tubulin III (*βTIII*), and *Nestin* gene levels were compared via reverse transcription polymerase chain reaction (RT-PCR) in these NSCs. Nestin and Gfap protein levels were compared by immunoassay. Subsequently, both populations were induced with 10^{-8} M selegiline for 48 hours, followed by immunohistochemical analysis of tyrosine hydroxylase (TH) levels. One-way ANOVA and Tukey's post-test were used with a significance level of $P<0.05$.

Results: Both groups were successfully expanded *in vitro* and expressed the neurotrophin receptor genes. The SGZ-NSCs had a significantly higher proliferation rate and significantly higher numbers of Nestin and Gfap-positive cells. Although the majority of selegiline-induced NSCs were TH-positive, we observed more TH-positive cells in SGZ-derived NSCs and these SGZ-NSCs displayed a shorter differentiation time.

Conclusion: SGZ-derived NSCs appear to be a more appropriate candidate for therapeutic purposes based on proliferation rate, neurosphere size, and *Gfap* and *Nestin* expression levels, as well as differentiation time and TH expression level after dopaminergic induction.

Keywords: Differentiation, Neural Stem Cells, Selegiline, Subgranular Zone, Subventricular Zone

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Introduction

Neurodegenerative diseases are the most important cause of death worldwide. The cost and impact on quality of life from these illnesses becomes more crucial because the risk of neurodegeneration increases with age. Rapid progress in stem cell therapy has led to an increased focus on this field. Cell therapy is a recently developed treatment that can compensate for defects and limitations of present chemical therapeutics and may be suitable for Parkinson's and Alzheimer's diseases (1, 2). Movement disorder is the main symptom of Parkinson's disease (PD) and it is caused by damage to dopaminergic neurons in the substantia nigra (1, 3). Adult neural stem cells (NSCs) exist in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) of the adult mammalian brain, where neurogenesis persists throughout life (4).

Research in animal models and clinical trials show that NSCs-based therapy is a promising tool to treat neurodegenerative diseases. NSCs can be transplanted without the need for genetic manipulation because they have the same embryonic origin as the host tissues (5-9). Stem cell-based therapies have garnered increased attention as a means for replacing these absent dopaminergic neurons and for restoration of motor function in patients who suffer from PD. Culture-expanded NSCs are multipotent can differentiate into many neuronal cell types; in addition, they secrete anti-inflammatory and neurotrophic factors. Features of NSCs that include integration into neural tissue, maintenance of homeostasis, and neuroprotective capability make them perfect candidates for stem cell therapy (10).

In this context, the selection of a suitable NSCs source

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may contribute to the survival, integration, and function of grafted cells (10, 11). Results of preclinical research show that the stimulated secretion of neurotrophic factors such as glial cell derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF) by adult stem cells results in a relatively large number of striatal tyrosine hydroxylase (TH)-positive neurons (12). Selegiline is one therapeutic agent for PD because of its anti-apoptotic and neuroprotective roles in hippocampal pyramidal cells and dopaminergic neurons (13). The neurotrophic effects of selegiline on neuronal survival and regeneration is mediated through neurotrophic factors (13, 14) such as NGF, CNTF, NT3, NT4/5, GDNF, BDNF, NGFR, tyrosine kinase receptor A (TrkA), and beta-tubulin III (βTIII). Neurotrophic factors are probably involved in the formation of the "stem cell niche", which preserves the self-renewal and multipotent state of cells in the adult brain (15, 16).

However, there are some concerns about this area of study. For example, an efficient and optimal culture condition that effectively polarizes NSCs towards functional dopaminergic neurons has not been introduced. Here, we aim to compare genetic expression levels of glial fibrillary acidic protein (*Gfap),* p75 neurotrophin receptor *(Ngfr), TrkA, βTIII*, and *Nestin* in cultured NSCs derived from the SGZ and SVZ regions of adult rats. Our second aim is to compare the differentiation potential of NSCs from these neural regions after co-treatment with selegiline.

Materials and Methods

Isolation and culture of neural stem cells

The Research and Ethics Committee of Damghan University, Damghan, Iran approved the experimental protocol of this study (2213/96). Adult male Wistar rats that weighed 200-250 g (Razi Institute, Karaj, Iran) were kept under standard laboratory conditions of a 12 hours light-dark cycle and ad libitum access to water and food. Under the dissecting stereomicroscope and using artificial cerebrospinal fluid and aseptic technique, the SGZ and SVZ regions of the rat brains were removed bilaterally from the anterior walls of the lateral ventricle and DG, respectively, and subsequently seeded as previously described (16). Of note, the SGZ and SVZ regions should be precisely dissected from other potential neurogenic areas. Corresponding to coronal coordinates interaural 4.48-5.86 mm, bregma -4.52 to bregma -3.14 (DG) and interaural 8.7-10.2 mm, bregma -0.30 to bregma -1.2 mm (SVZ). Briefly, the SGZ and SVZ tissues were mechanically and enzymatically dissociated with 2 ml of 0.1% trypsin-EDTA (Sigma Aldrich, NSW, Australia). After 20 minutes, we added trypsin inhibitor and the solution was manually triturated. The solution was centrifuged at 500×g for 5 minutes and we aspirated the supernatant. Next, we used a glass Pasteur pipette to mechanically dissociate the resultant cell pellet 25 times in 3 ml medium and 20 times with 1 ml pipette tips to ensure that all of the cells were well-dispersed. We used the Neubauer method to subsequently count the cells in this suspension. Then, the cells were allowed to proliferate in α-minimal essential medium (α-MEM) supplemented with 1% penicillin/streptomycin (Gibco, USA), 10% fetal bovine serum (FBS), 20 ng/ml basic fibroblast growth factor (bFGF), 20 ng/ml epidermal growth factor (EGF) and B27 supplement at 37°C and 5.0% CO_2 for six days. Every three days, we changed 50% of the medium. When cells reached 80% confluency, they were sub-cultured (days 8-9) in a 0.25% trypsin (Sigma, USA) and 0.04% EDTA (Sigma, St. Louis, MO, USA) solution.

Cell proliferation rate assays

The SGZ-derived NSCs and SVZ-derived NSCs were incubated in 10 µm bromodeoxyuridine (BrdU, Sigma) for 24 hours. For the BrdU-labelling assay, the cells were fixed with 70% ethanol for 10 minutes and then exposed to 2 M of HCl for 1 hour at 37°C. Subsequently, the cells were incubated in 0.1 M of borate buffer (pH=8.5) for 10 minutes and placed in a blocking solution that consisted of phosphate-buffered saline (PBS), 10% goat serum, and 0.3% Triton X-100 at room temperature for 30 minutes. Next, the cells were exposed to mouse anti-BrdU primary antibody (1:500; B 2531; Sigma, St. Louis, MO, USA) followed by anti-mouse secondary antibody conjugated with peroxidase (1:200; A 2304; Sigma, St. Louis, MO, USA). We calculated the number of nuclei of the positive cells in 20 fields. The negative control consisted of normal goat serum, which was replaced with the primary antibodies. Each experiment was repeated five times (17).

Reverse transcription polymerase chain reaction

Total RNA was extracted from passage-3 NSCs using an RNX-Plus kit (Cinnagen, Iran). The quality of the RNA was established by agarose gel electrophoresis. We used 0.5 µg of total RNA to perform cDNA synthesis according to the manufacturer's protocol with the RevertAid™ First Strand cDNA Synthesis kit (#k1622; Fermentas, USA). *Gfap*, *Ngfr*, *TrkA*, *βTIII*, and *Nestin* expression levels were measured by reverse transcription polymerase chain reaction (RT-PCR) using a master cycler (Eppendorf, Hamburg, Germany). The PCR mix consisted of 5 µg of synthesized cDNA, 1x PCR buffer, 50 mM $MgCl_2$, 10 mM dNTPs, 10 pmol forward and reverse primers and 0.25 µl Taq DNA polymerase in a final volume of 25 µl. The PCR protocol comprised: 2 minutes at 94°C; 34 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C; and 5 minutes of a terminal extension at 72°C. Beta-2 microglobulin (*β2M*) was utilized as the housekeeping gene (internal control). Subsequently, the PCR products were loaded on 1.5% agarose gel for adequate separation, then visualised and photographed under a UV transilluminator (UVIdoc, Uvitec, UK). We used UVIdoc software

(version 12.6) for quantitative assessment of the band sizes. Each experiment was conducted in triplicate. Negative controls used for the cDNA preparation stage included minus RNA (-RNA) and minus reverse transcriptase (-RT). RT-PCR analyses were checked by using two negative controls, no Taq polymerase [no amplification control (NAC)] and no cDNA templates (-cDNA control) (18).

We used the following primer sequences:

Gfap-

F: 5′- ACCTCGGCACCCTGAGGCAG -3′
R: 5′- CCAGCGACTCAACCTTCCTC -3′

Ngfr-

F: 5′- ACGACCAGCAGACCCATA -3′
R: 5′- GGTATCCCCGTTGAGCAGT -3′

TrkA-

F: 5′- GCTGAGTGCTACAACCTTCTGA -3′
R: 5′- GAAGCGTACGATGTGTTGGT -3′

βTIII-

F: 5′- TGCGTGTGTACAGGTGAATGC -3′
R: 5′- AGGCTGCATAGTCATTTCCAAG -3′

Nestin-

F: 5′- TGGTGAGGGTTGAGGTTTGT3 -3′
R: 5′- CAGGCTTCTCTTGGCTTTCTGG -3′

β2M-

F: 5′- CCGTGATCTTTCTGGTGCTT -3′
R: 5′- TTTTGGGCTTCAGAGTG -3′

Immunostaining of the neural stem cells for Nestin and glial fibrillary acidic protein

We used anti-nestin and anti-Gfap immunostaining to identify the stemness of the NSCs. Glass coverslips were coated with 50 µg/ml poly-D-lysine (Sigma, St. Louis, MO, USA) and a concentration of 5×10^4 cells/cm^2 cells was added for to immunocytochemistry analysis according to standard protocols. Briefly, the cells were fixed with 4% paraformaldehyde at room temperature for 20 minutes and rinsed three times in PBS. Next, as a required step for Nestin and Gfap protein analysis, the cells were permeabilized by 0.3% Triton X-100 in PBS for 15 minutes and 10% goat serum at room temperature for 15 minutes, and then incubated overnight at 4°C with special antibodies directed against the different phenotypic markers. The primary antibodies used were anti-Gfap (1:100; G6171; Sigma, St. Louis, MO, USA) and anti-nestin (1:500; N5413; Sigma, St. Louis, MO, USA). Then, the cells were washed twice in PBS and stained with fluorescein isothiocyanate (FITC)-conjugated secondary anti-mouse (1:100) for Nestin and rhodamine-conjugated secondary anti-rabbit (1:100) for Gfap (AP124R; Millipore, USA) at 37°C for 1 hour. The stained cells were observed under a fluorescent microscope (E600 Eclipse; Nikon) equipped with a digital camera (DXM 1200; Nikon Europe BV, The Netherlands). The primary antibody was eliminated to prevent false positive reactions. The immunocytochemical experiments were repeated thrice (19, 20).

Differentiation of subgranular zone-derived neural stem cells and subventricular zone-derived NSCs into dopaminergic neurons

Passage-3 cells were placed on 12-well plates and covered with 50 µg/ml poly-D-lysine (Sigma, St. Louis, MO, USA) at a density of 10^4 cells/cm^2. Once the viable seeded cells had adhered to the bottom of the plate, we removed the supernatant and replaced it with an induction medium that consisted of serum-free α-MEM and 10^{-8} M selegiline for 24 hours. Then, the cells were incubated in serum-free α-MEM without inducers for 24 hours. After 48 hours (24 hours with selegiline and 24 hours without selegiline), the induced cells were used for immunocytochemistry analysis to assess the level of the neural-specific proteins (13, 21, 22). TH immunocytochemistry was carried out as previously described. Briefly, the cultured cells were fixed with 70% ethanol for 10 minutes and then rinsed with 0.1 M of borate buffer (pH=8.5) and PBS. These cells were subsequently incubated in 0.3% Triton X-100 that contained 10% goat serum. After 24 hours incubation with primary antibody against TH followed by exposure to horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hour, the cells were incubated with 3,3-diaminobenzidine (DAB, Sigma, St. Louis, MO, USA) for 20 minutes. Microscopic analysis was performed after dehydration and clearing. The slides were photographed using a digital camera (DXM 1200; Nikon Europe BV, The Netherlands) and analysed by ImageJ software.

Statistical analysis

We used one-way ANOVA and Tukey's supplementary test with a significance level of $P<0.05$ to compare the experimental groups. Statistical values were calculated using SPSS version 26 software package (IBM, USA).

Results

Morphological characterizations of the subgranular zone neural stem cells and and subventricular zone NSCs

We cultured the adult rat SGZ- and SVZ-derived NSCs in α-MEM supplemented with 10% FBS, 20 ng/ml bFGF, 20 ng/ml EGF and B27 supplement. Our primary culture contained long bipolar cells, round cells, and cells with extensive neurite outgrowth.

The cell viability was approximately 95% in all groups.

Morphological analysis results showed the presence of adhesive and semi-adhesive neurospheres in both types of NSCs. There were a few neurospheres with variable sizes in the first passage [20-30 µm diameter (arrowheads), 30-40 µm (thin arrows)] and in the second passage (40-50 µm; bold arrows) (Fig.1A). The SGZ group had significantly larger neurospheres compared with the SVZ group ($P<0.05$, Fig.1B).

Bromodeoxyuridine analysis of cell proliferation rate

MTT and Bromodeoxyuridine (BrdU) labelling analysis indicated that the proliferation rate of SGZ-derived NSCs was higher than SVZ-derived NSCs (MTT results not shown). There was a quantitative increase in the proliferation rate of SGZ-derived NSCs at 48 hours compared with SVZ-derived NSCs (Fig.2).

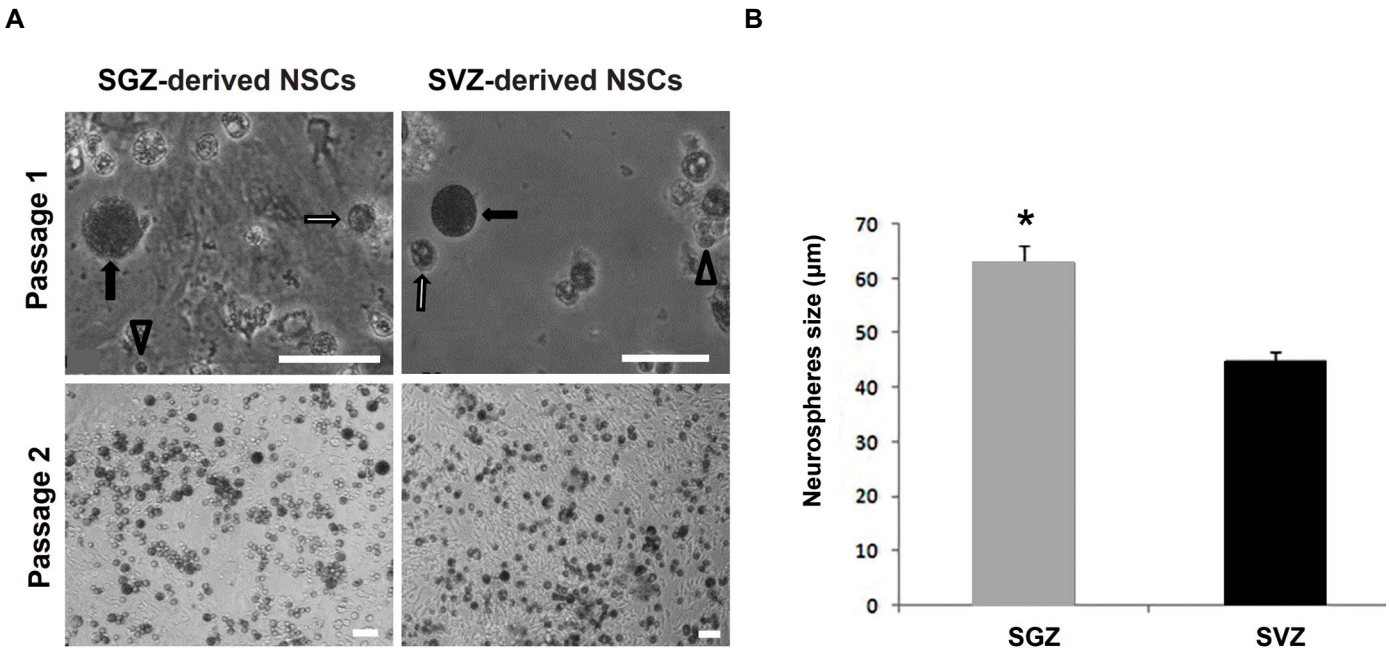

Fig.1: Microscopic observation of neurospheres derived from the SGZ-derived NSCs and SVZ-derived NSCs. **A.** Neurospheres are derived from passages 1 and 2 SGZ-derived NSCs and SVZ-derived NSCs. **B.** Comparison of the sizes of the neurospheres generated from the monolayer culture condition of passage-1 SGZ-derived NSCs and SVZ-derived NSCs in α-MEM enriched with 10% FBS, 20 ng/ml bFGF, 20 ng/ml EGF and B27 supplement. Neurospheres derived from the SGZ are significantly larger than those from SVZ-derived NSCs (scale bar: 50 µm). SGZ; Subgranular zone, NSCs; Neural stem cells, SVZ; Subventricular zone, α-MEM; α-minimal essential medium, FBS; Fetal bovine serum, bFGF; Basic fibroblast growth factor, EGF; Epidermal growth factor, and *; $P<0.05$.

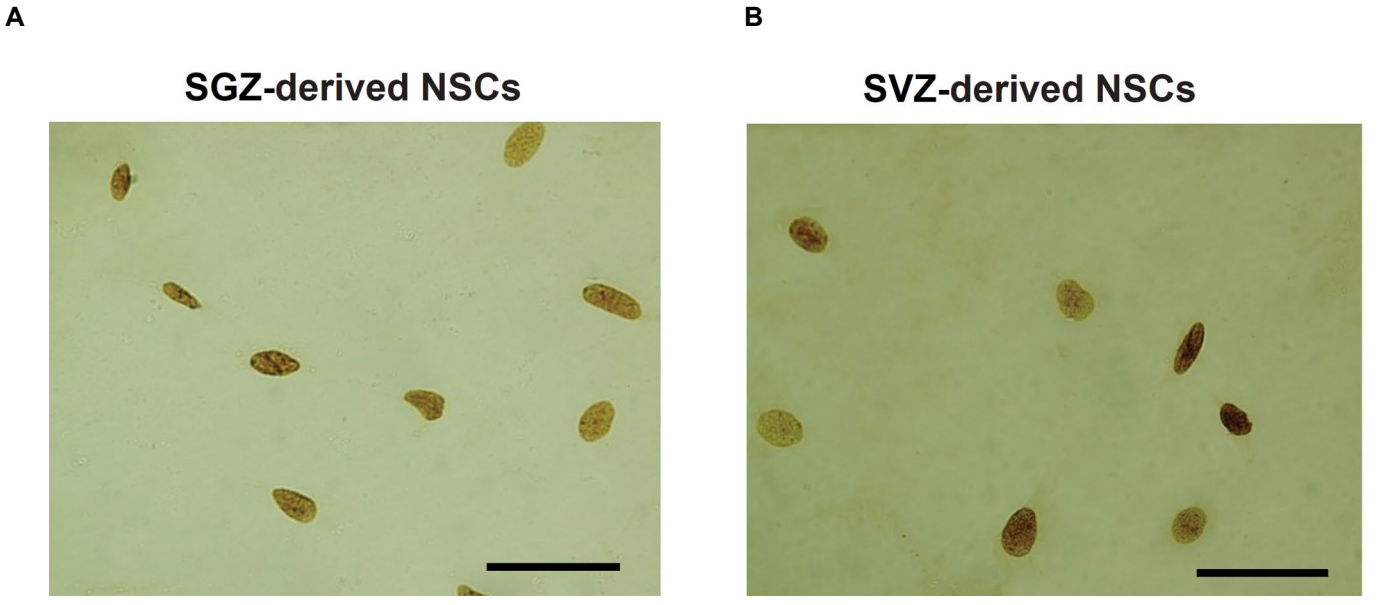

Fig.2: Immunocytochemical staining for BrdU in SGZ-derived NSCs and SVZ-derived NSCs. BrdU incorporation assay evaluation of the proliferation capabilities of: **A.** SGZ-derived NSCs and **B.** SVZ-derived NSCs (scale bar: 50 µm). BrdU; Bromodeoxyuridine, SGZ; Subgranular zone, NSCs; Neural stem cells, and SVZ; Subventricular zon.

Gene expression analysis

Figure 3 shows the RT-PCR results of the *Gfap*, *Ngfr*, *TrkA*, *βTIII*, and *Nestin* genes in the SGZ- and SVZ-derived NSCs. The results of the relative expressions were normalized to β2-microglobulin mRNA amplification with a statistical significance of $P<0.05$ for all of the compared groups. Although we observed significantly higher *Nestin* expression in the neurospheres compared to the control gene, the RT-PCR data did not detect any significant difference in expression patterns of these genes between the SGZ- and SVZ-derived NSC groups.

Identification of the subgranular zone- and subventricular zone-derived neural stem cells by Nestin and glial fibrillary acidic protein immunocytochemistry analysis

Phase-contrast and fluorescence images showed mostly bipolar nestin-positive cells with stretched cytoplasms. Multipolar cells with large nuclei in the form of round to oval shapes were occasionally observed. The numbers of nestin-positive SGZ- and SVZ-derived NSCs were 82.50 ± 0.645% and 79.75 ± 0.479%, respectively (Fig.4). We observed a significant difference between the experimental groups ($P<0.05$). There was a significant difference ($P<0.05$) between the Gfap-positive SGZ-derived NSCs (11.50 ± 0.041%) and SVZ-derived NSCs (5.75 ± 0.854%) (Fig.5). Overall, in our adherent model of cultured NSCs, the expression levels of the Gfap and Nestin proteins in the SGZ-derived NSCs were significantly higher than in the SVZ-derived NSCs (Figs.4, 5).

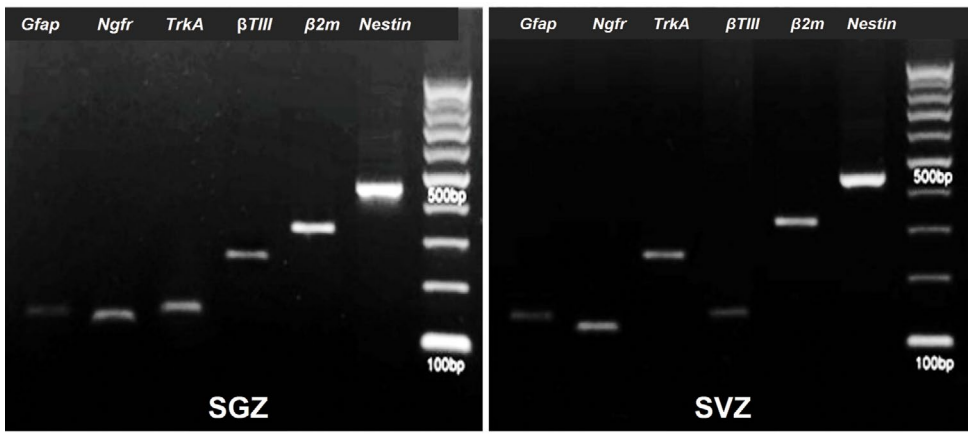

Fig.3: Analysis of gene expressions in SGZ-derived NSCs and SVZ-derived NSCs for NSC specific markers. Agarose gel electrophoresis of RT-PCR products of neural-specific genes: *Gfap*, *Ngfr*, *TrkA*, *βTIII*, *B2M*, and *Nestin* in SGZ-derived NSCs and SVZ-derived NSCs. RT-PCR results show that the genes have similar expression patterns between SVZ-derived NSCs and SGZ-derived NSCs. SGZ; Subgranular zone, NSCs; Neural stem cells, SVZ; Subventricular zone, and RT-PCR; Reverse transcription polymerase chain reaction.

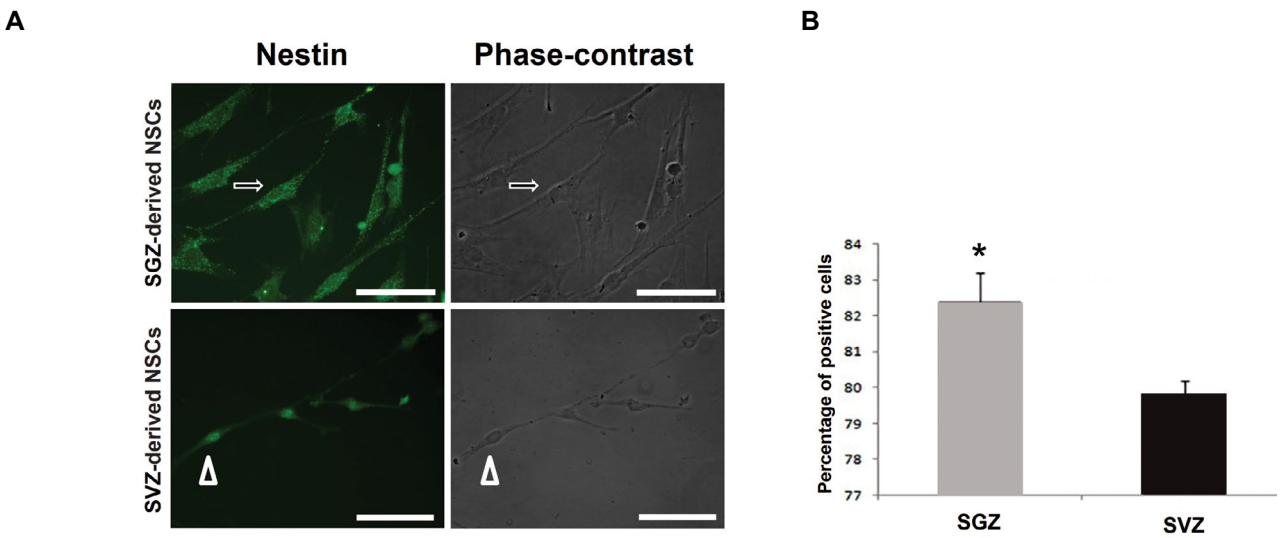

Fig.4: Immunocytochemical staining for antibody Nestin protein in neurospheres derived from the SGZ-derived NSCs and SVZ-derived NSCs. **A.** Fluorescence and phase-contrast images of the SGZ-derived NSCs (thin arrows) and SVZ-derived NSCs (arrowheads) for the Nestin marker. Nestin immunostaining by FITC-conjugated secondary antibody (green colour). **B.** Percentages of nestin-positive SGZ-derived NSCs (82.50 ± 0.645%) and SVZ-derived NSCs (79.75 ± 0.479%) are shown. There is a significant difference between these two groups (scale bar: 50 µm). SGZ; Subgranular zone, NSCs; Neural stem cells, SVZ; Subventricular zone, FITC; Fluorescein isothiocyanate, and *; $P<0.05$.

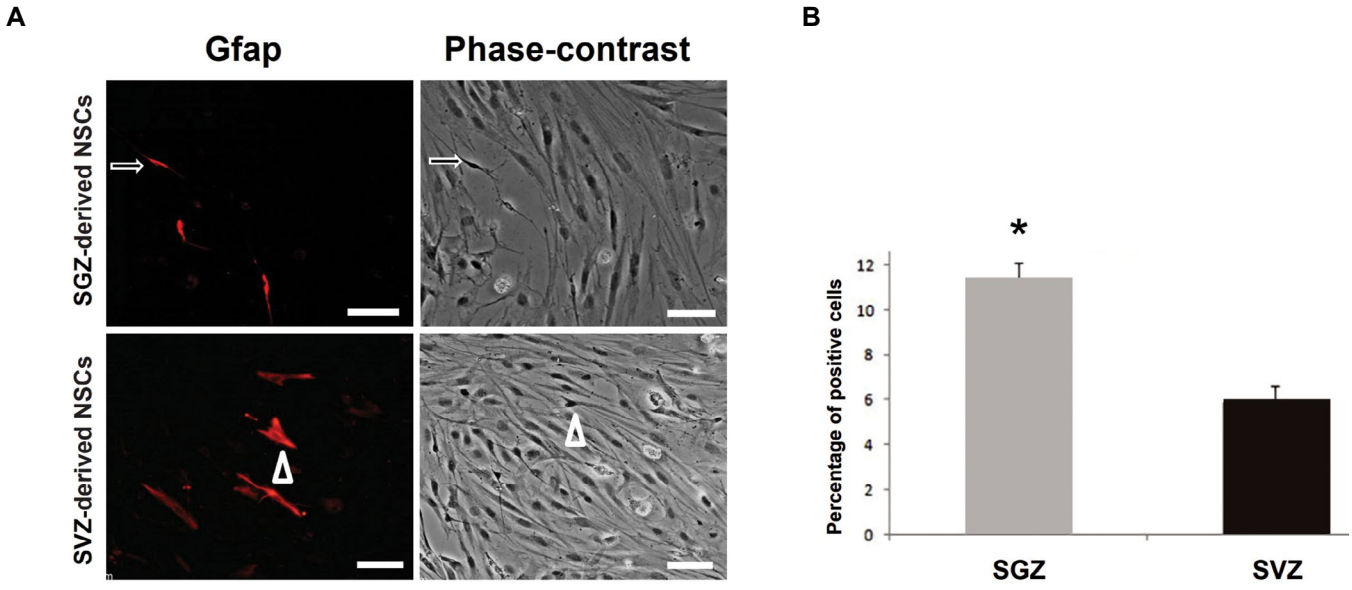

Fig.5: Immunocytochemical staining for antibody Gfap in neurospheres derived from the SGZ-derived NSCs and SVZ-derived NSCs. **A.** Fluorescence and phase-contrast images of the SGZ-derived NSCs (thin arrows) and SVZ-derived NSCs (arrowheads) for the Gfap marker. Gfap immunostaining by rhodamine-conjugated secondary antibody (red colour). **B.** There is a significant difference between Gfap-positive SGZ-derived NSCs (11.50 ± 0.041%) and SVZ-derived NSCs (5.75 ± 0.854%) (scale bar: 50 µm). SGZ; Subgranular zone, NSCs; Neural stem cells, SVZ; Subventricular zone, and *; P<0.05.

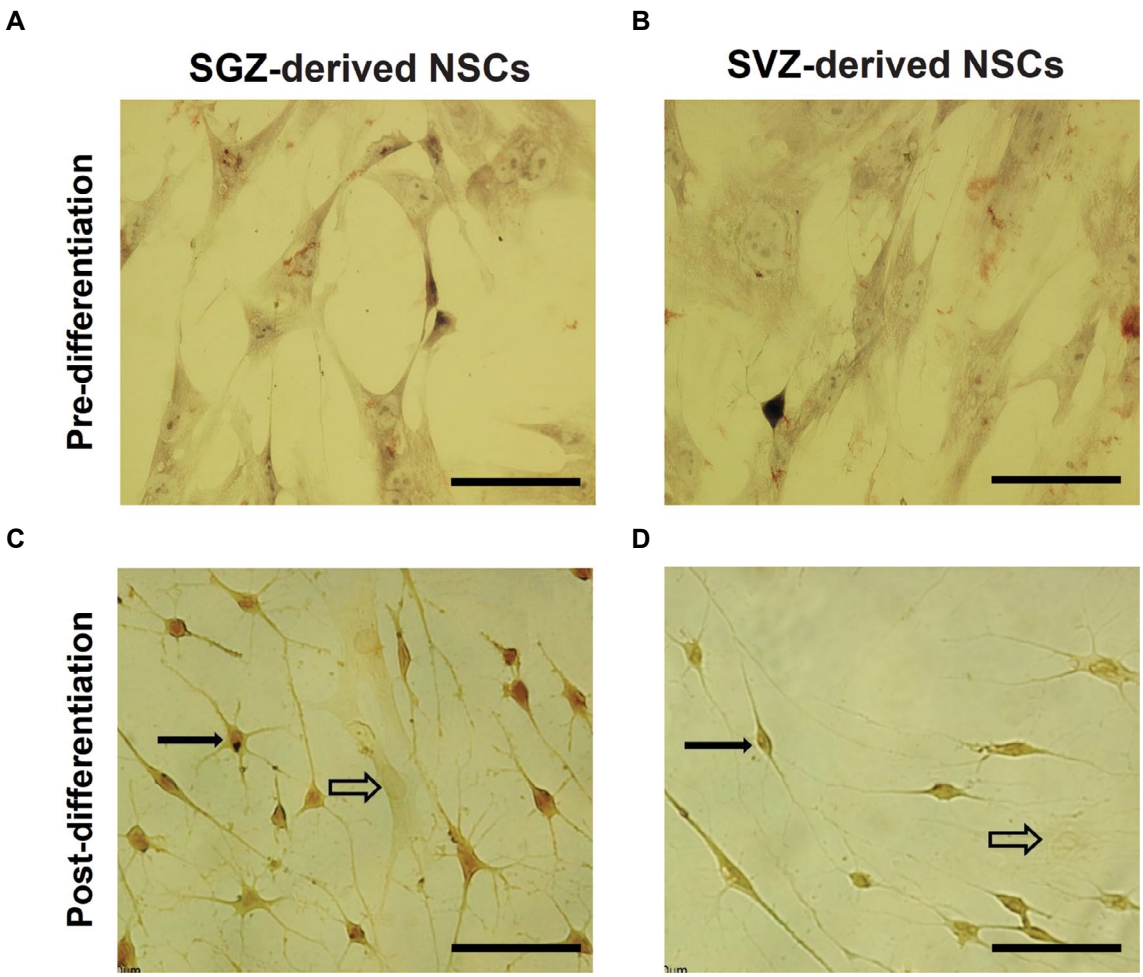

Fig.6: Immunocytochemical staining for TH in SGZ-derived NSCs and SVZ-derived NSCs. **A, B.** Anti-TH immunostaining before selegiline induction. **C, D.** Anti-TH immunostaining after selegiline induction. C, D show the presence of TH-positive dopaminergic-like neurons visualized after staining with DAB for light microscope observation. The majority of SGZ-derived NSCs and SVZ-derived NSCs are TH positive (scale bar: 50 µm). TH; Tyrosine hydroxylase, SGZ; Subgranular zone, NSCs; Neural stem cells, SVZ; Subventricular zone, and DAB; 3,3-diaminobenzidine.

Tyrosine hydroxylase immunostaining

Passage-3 cultured NSCs were incubated for 48 hours in neuronal differentiation medium that contained selegiline. TH immunostaining results showed that none of the cells in the groups expressed TH prior to differentiation. Nevertheless, neuron-like morphological changes and altered cytoplasmic processes were observed after neuroinduction with selegiline. The cells acquired a bipolar morphology with neuron-like cell processes during neuroinduction; afterwards, most NSCs showed multipolar morphologies with neural processes. Statistically higher levels of TH were detected in SGZ-derived NSCs and neuron-like morphology appeared earlier in this group compared with SVZ-related NSCs (Fig.6).

Discussion

Here, we investigated several features of adherent cultured NSCs that have particular significance in regenerative medicine. To the best of our knowledge, this is the first study to compare the characteristic features of rat SGZ-derived NSCs and SVZ-derived NSCs *in vitro*. Our observations indicated dopaminergic differentiation of SGZ-derived NSCs and SVZ-derived NSCs in the selegiline-enriched neuroinduction medium.

Selegiline is a monoamine oxidase B inhibitor that can limit degradation of the neurotransmitter dopamine at central nervous system (CNS) synapses, thereby protecting dopaminergic neurons. The effect of selegiline on differentiation of NSCs into neuron-like cells has been well studied (23-25). The results of some studies suggest a therapeutic efficacy of selegiline for the treatment of neurodegenerative diseases (26). Our data reveal that selegiline has an excessive potential as an agent to stimulate the differentiation of NSCs towards dopaminergic neurons in neuroinduction medium. We propose that the beneficial effects of selegiline are more evident in SGZ-derived NSCs and this can indicate a higher potential of this neuronal stem cell niche to be used in the management of PD.

In this research, SGZ-derived NSCs and SVZ-derived NSCs were cultured on uncoated plastic plates as a monolayer of the adult mouse brain for extended passages. We observed neurosphere masses in the first and second passages of our primary culture. The total number and size of the SGZ-derived neurospheres were significantly higher than the SVZ-related neurospheres. On the other hand, we noted that more than 90% of the NSCs were BrdU positive.

Gene expression results revealed that the SGZ-derived NSCs and SVZ-derived NSCs had similar expression patterns for *Gfap*, *Ngfr*, *TrkA*, *βTIII*, and *nestin*. Nestin and Gfap are considered markers of neural stem/progenitor cells and astrocyte cells, respectively (27). However, NSCs extracted from neurogenic regions can be identified by the amounts of Nestin and Gfap proteins (28, 29). In the current study, we found that 82.50 ± 0.645% of SGZ-derived NSCs and 79.75 ± 0.479% of SVZ-derived NSCs expressed nestin, whereas 11.50 ± 0.041% of SGZ-derived NSCs and 5.75 ± 0.854% of SVZ-derived NSCs expressed Gfap. Therefore, our immunocytochemistry data showed that the majority of these cultured neural progenitors expressed a neuronal marker (nestin) and a small percentage expressed an astrocyte-specific marker (Gfap). Previous studies have shown similar results (30).

Neurotrophic factors such as NGF, NT3, NT5, GDNF, and BDNF are critical during central connection, regulation of neuronal survival, and protection of transplanted cells (31, 32). The results of *in vitro* studies have reported that exposure to D2 dopamine agonists can induce the synthesis/secretion of important neurotrophic factors such as GDNF, NGF, and BDNF (33).

In this study, RT-PCR analysis results showed a similar gene expression pattern in the NSCs derived from both neurogenic regions. Although we did not investigate the expression level of nerve growth factors in this study, we examined the expression levels of two important nerve growth factor receptors, *TrkA* and *Ngfr. TrkA* is a receptor with kinase properties. Along with *Ngfr,* it mediates multiple effects of NGF such as neuronal differentiation, neuronal proliferation, and prevention of programmed cell death. We found that the expression of the protein kinase receptors *TrkA* and *Ngfr* did not change significantly in NSCs derived from both groups. NSCs are self-renewing and multipotent cells that provide neurotrophic factors to support endogenous precursor cells of the adult brain.

The immunostaining findings of neural stem and progenitor cells in other studies has shown the presence of nestin-positive cells in both neurosphere and adherent cultures (34). We observed a high expression level of Nestin based on immunocytochemistry and RT-PCR results. Therefore, a monolayer culture of NSCs in the presence of serum without growth factors was found to be a desirable culture condition that had the capacity to regulate proliferation and differentiation.

Our previous study results have shown that PD is typically managed pharmacologically by increasing the production of dopamine in surviving dopaminergic neurons. The TH marker was used to demonstrate the dopaminergic neurons *in vitro* (2). Also, in this study, it was demonstrated that selegiline is a direct inducer that can differentiate NSCs into dopaminergic-like neurons. This culture system can be used to expand NSCs for stem cell studies and cell therapy, and provides a good *in vitro* system to model *in vivo* proliferation.

Our study had some limitations. We did not investigate the mechanism involved in selegiline-induced NSCs differentiation, nor did we investigate the differentiation effect of selegiline *in vivo*. Overall, the upper proliferation and differentiation capabilities of SGZ-derived NSCs compared with SVZ-derived NSCs provides a suitable cell source for stem cell therapy in neurodegenerative disorders that are affected by degeneration of dopaminergic neurons.

Conclusion

Selegiline induced the differentiation of SGZ-derived NSCs and SGZ-derived NSCs into dopaminergic neurons; this differentiation effect of selegiline appears to be more effective in cultured SGZ-derived NSCs.

Acknowledgments

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Authors' Contributions

M.Gh.; Writing, Original draft preparation, and Investigation. L.M.; Software, Reviewing, Editing, and Methodology. M.T.Gh.; Supervision, Conceptualization, and Methodology. F.R.; Reviewing, Editing, and Software. All authors read and approved the final manuscript.

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