Exogenous Expression of Nt-3 and TrkC Genes in Bone Marrow Stromal Cells Elevated the Survival Rate of the Cells in the Course of Neural Differentiation

Article *in* Cellular and Molecular Neurobiology · January 2019

CITATION
1

READS
35

1 author:

Houri Edalat
Baqiatallah University of Medical Sciences
14 PUBLICATIONS **70** CITATIONS

SEE PROFILE

Cell Mol Neurobiol
DOI 10.1007/s10571-016-0448-y

ORIGINAL RESEARCH

Exogenous Expression of *Nt-3* and *TrkC* Genes in Bone Marrow Stromal Cells Elevated the Survival Rate of the Cells in the Course of Neural Differentiation

Houri Edalat[1] · Zahra Hajebrahimi[2] · Vahid Pirhajati[3] · Mahmoud Tavallaei[1] · Mansoureh Movahedin[4] · Seyed Javad Mowla[5]

Received: 20 January 2016 / Accepted: 4 May 2016

Abstract Bone marrow stromal cells (BMSCs) are attractive cellular sources for cell therapy of many diseases, specifically neurodegenerative ones. The potential capability of BMSCs could be further augmented by enhancing their neuroprotective property, differentiation potential, and survival rate subsequent to transplantation. Therefore, a concurrent upregulation of neurotrophin-3 (*NT-3*) and its high affinity receptor, tyrosin kinase C *(TrkC)*, was utilized in our study. BMSCs were cotransfected with pDsRed1-N1-NT-3 and pCMX-TrkC plasmids before induction of neural differentiation. pEGFP-N1-transfected BMSCs were also employed as a control. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was employed for gene expression analysis. Cell viability was evaluated by MTT assay, while apoptosis rate was assessed by flow cytometry after PI and Annexin V staining. NT-3 and TrkC mRNA levels were greatly elevated following cotransfection of cells with pDsRed1-N1-NT-3 and pCMX-TrkC vectors. The expression of neural markers (i.e., NFM, and NeuroD1) was augmented in cotransfected BMSCs, compared to the control ones, after neural induction. At each time point, the viability and apoptosis rates of the cells over-expressing *NT-3* and *TrkC* showed increased and reduced patterns, respectively. Our data demonstrated that *NT-3*/*TrkC*-co-transfected BMSCs, compared to those of intact cells, could be more beneficial graft candidates for the upcoming treatment strategies of neurogenic disorders due to their increased viability and expression of neural markers. This may be due to their increased level of neural differentiation potential and/or their enhanced rate of survival and/or their useful capacity to secrete NT-3.

Keywords Bone marrow stromal cells · *NT-3* · *TrkC* · Apoptosis · Cell viability · Neural differentiation

Introduction

Adult or somatic stem cells are readily able to replace and repair the damaged tissues in the body. As remaining self-renewal and pluripotency traits, adult stem cells do not encounter tumorigenicity or ethical problems attributed to embryonic-derived cells. From among adult stem cells, bone marrow stromal cells (BMSCs) have been introduced as one of the most attractive sources for cell therapy of various diseases including neurological ones. BMSCs can be easily obtained from the patient for autologous transplantation. They can also modulate the immune system through suppressing the functions of T, B, and NK cells. The last two mentioned features circumvent rejection problems and the need for immunosuppressive therapies that are tackled with in other kinds of transplanting cells.

Electronic Supplementary Material The online version of this article (doi:10.1007/s10571-016-0448-y) contains supplementary material, which is available to authorized users.

✉ Seyed Javad Mowla
sjmowla@modares.ac.ir

[1] Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

[2] Department of Physiology, Aerospace Research Institute, Tehran, Iran

[3] Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

[4] Department of Anatomy, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[5] Department of Molecular Genetics, School of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Published online: 28 November 2016

BMSCs have an innate tropism for sites of illness in the body. They also provide a proven safety record in hematological malignancies for several decades (Caplan 2007; Hokari et al. 2008).

Neurotrophin-3 (*NT-3*), nerve growth factor (*NGF*), as well as brain derived neurotrophic factor (*BDNF*) are three important members of neurotrophin family. NGF, BDNF, and NT-3 specifically interact with their specific high affinity cognate receptors called Tyrosin kinase A (TrkA), TrkB, and TrkC, respectively. The specific binding of neurotrophins to their receptors initiates a number of biological effects, including axon regeneration, neuronal survival, re-myelination augmentation, and stimulating endogenous stem cells in both of in vitro and in vivo conditions (Blesch 2006).

Based on our former observations, BMSCs express *NGF* and *BDNF* both before and after neural differentiation, while *TrkA* and *TrkB* are expressed only following neural induction. On the other hand, these cells did not express *NT-3* and *TrkC* either before or after differentiation (Yaghoobi and Mowla 2006). Therefore, the aim of our study was concurrent over-expression of both *NT-3* and *TrkC* in BMSCs to increase efficiency of survival, synthetic activity, and even differentiation potential of the cells after grafting.

Materials and Methods

Cell Culture and Transfection

BMSCs were extracted from Sprague–Dawley rats (Pasteur Institute, Tehran, Iran) and transfected by Lipofectamine 2000 (Invitrogen, USA) as described previously (Edalat et al. 2011, 2013; Yaghoobi et al. 2005). Efforts were made to reduce the pain and the number of investigated animals. Cotransfection was optimized by using a gradient concentration containing half, equal, twice, and four times of the amount recommended by the manufacturer for each of pDsRed1-N1-NT-3 and pCMX-TrkC vectors.

Then, BMSCs were cotransfected with pDsRed1-N1-NT-3 and pCMX-TrkC plasmids (kindly provided by Professor Philip A. Barker, Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada) and the pEGFP-N1 plasmid (used as a control).

Restriction enzyme digestion, PCR, and DNA sequencing were used to verify the presence or integrity of *NT-3* and *TrkC* cDNAs which were cloned in aforementioned vectors. Sequences of primers used for PCR are provided elsewhere (Hajebrahimi et al. 2008; Yaghoobi and Mowla 2006).

Neural Induction, RNA Extraction, and cDNA Synthesis

Cells cotransfected with pDsRed1-N1-NT-3 and pCMX-TrkC (*NT-3*/*TrkC*) along with pEGFP-N1-transfected cells (mock) were induced for neural differentiation according to previous descriptions at about 24 h after transfection (Edalat et al. 2011). Briefly, a pre-differentiation was performed by adding pre-induction medium containing 20% FBS (Invitrogen, USA) and 10 ng/ml bFGF (Roche, Germany) for 24 h. Then, the pre-induction medium was replaced with neural induction medium on the next day (Edalat et al. 2011).

The cells were monitored continually after neuronal induction and were lysed for RNA extraction or subjected to assays at specific time points. An un-induced parallel culture dish was also analyzed along with every experiment as a control. Total RNA extraction and cDNA synthesis were performed using High Pure RNA Isolation (Roche, Germany) and Prime Script^{TM} 1st strand cDNA Synthesis (Takara, Japan) kits, respectively, as said by recommended instructions. DNase I treatment was included in the RNA extraction process. The quality of RNA was assessed by gel electrophoresis and Nanodrop (Thermo scientific, USA) methods. RNA quantity was also measured by Nanodrop at 260 nm. 100 ng RNA was used in a final volume of 20 µL cDNA synthesis reaction. Reverse Transcriptase was finally inactivated by incubating at 85 °C for 5 s.

Primer Design and Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Primers were designed using Allele ID 6.0 software and then submitted to BLAST search to ensure that the sequences were specific just for the gene of interest (Table 1).

Real-time qRT-PCR reactions were performed on an ABI 7500 real-time quantitative PCR system using SYBR Green Master mix (Ex Taq II) (Takara, Japan) with 5 µl master mix, 0.2 µM forward primer, 0.2 µM reverse primer, 0.04 µl ROX reference dye II, 1 µl cDNA Template, and dH2O to a final volume of 10 µl in the following cycling conditions: initiation at 94 °C for 30 s, amplification for 40 cycles with denaturation at 94 °C for 5 s and annealing and extending at 60 °C for 34 s.

LinReg PCR software was utilized to determine the reaction efficiencies for each primer pair. All experiments were conducted in duplicate or triplicate. Group-wise comparison and statistical analysis of relative expression results of real-time qRT-PCR were carried out by REST 2008 software. SPSS 17.0 for windows was also used to

Table 1 List of primers employed in this study

Gene	Ref seq	Function	Primer sequence (5′ → 3′)	bp
β2 m	NM_012512.1	Housekeeping	F: CGTGATCTTTCTGGTGCTTGTCTC	151
			R: TCTATCTGAGGTGGGTGGAACTG	
Gapdh	NM_017008.3	Housekeeping	F: TGTGACTTCAACAGCAACTCCCAT	206
			R: CTCTCTTGCTCTCAGTATCCTTGC	
Actb	NM_031144.2	Housekeeping	F: CTGTGCTATGTTGCCCTAGACTTC	112
			R: CATTGCCGATAGTGATGACCTGA	
Ngf (NGF)	XM_227525.5	Neurotrophin	F: CACCTCTTCGGACACTCTGGA	166
			R: CGTGGCTGTGGTCTTATCTCC	
Bdnf (BDNF)	NM_012513.3	Neurotrophin	F: GTGACCTGAGCAGTGGGCAAAG	150
			R: ATATAGCGGGCGTTTCCTGAAGC	
Ntf-3 (NT-3)	NM_031073.2	Neurotrophin	F: CTGTGGGTGACCGACAAGTC	217
			R: AAGTCAGTGCTCGGACGTAGG	
Ntrk1 (TrkA)	NM_021589.1	Neurotrophin receptor	F: ATACCTGTGTCCACCATATCAAGC	166
			R: CGAGCATTCTCAGATGTCTCCTTC	
Ntrk2 (TrkB)	NM_012731.2	Neurotrophin receptor	F: TTATGCTTGCTGGTCTTGGGCTTC	146
			R: TCTGGGTCAATGCTGTTAGGTTCC	
Ntrk3 (TrkC)	NM_019248.1	Neurotrophin receptor	F: ACTTGTAATGGCTCTGGCTCTCC	145
			R: TGTCTTCGCTCGTCACATTCAC	
Ngfr ($p75^{NTR}$)	NM_012610.2	Apoptosis	F: CAACGGTCAGAACGGAGCATC	98
			R: AGAGGGTGGTCAGAAGCAAGG	
Nefm (NFM)	NM_017029.1	Neural marker	F: ACAGCCCTCAGTCACAATATCCA	104
			R: TAGTCTCCTCAATGATCTCCTCCA	
Map2 (MAP2)	NM_013066.1	Neural marker	F: CAGAACATACCACCAGCCCTTTG	110
			R: GTCTTTCCTCTCGTCAGCCATCC	
Neurod1	NM_019218.2	Neural marker	F: ACGCAGAAGGCAAGGTGTCC	108

analyze the correlation between the expressions of each gene in the cells at various time points.

At first, a primary analysis was performed by comparing gene expression in each of pEGFP-N1-transfected (as a control) and *NT-3*/*TrkC*-cotransfected cells at different time points of 0, 6, 12, and 24 h after neural induction. A secondary analysis of gene expression was then executed by comparing control and *NT-3*/*TrkC*-cotransfected cells at each of the aforementioned time points.

Examination of Cell Viability and Apoptosis

Cell viability and apoptosis assays were performed around 5–6 h after beginning of neural induction.

Cell viability was determined by using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, according to the manufacturer's instructions. 20 µl of 10 mg/ml MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma,USA) was added to 5×10^4 cells cultured in 96 flat-bottom well plates in a final volume of 200 µl. The cells were incubated for 3 h at 37 °C. Then 200 µl of dimethyl sulfoxide was introduced, as the solvent reagent. The amount of formazan produced from MTT cleavage was quantitated with an ELISA plate reader (BioTech Company, USA), at 490 nm wavelength.

Cell death was quantified by Annexin-V-FLUOS staining apoptosis detection kit (Roche, Germany), based on the company's protocol. Briefly, cells were harvested by trypsinization and then labeled with annexin V and propidium iodide (PI), before being analyzed by flow cytometry (Becton–Dickinson, USA). Dead cells were scored as necrotic (PI-positive/annexin V-negative) or apoptotic (annexin V-positive/PI-negative and annexin V positvie/PI-positive) (Edalat et al. 2011).

Cell-Cycle Analysis

Study of the cell cycle was also performed around 5–6 h after beginning of neural induction.

24 h after transfection (in non-differentiated cells), flow cytometry analysis was carried out as described elsewhere (Jafarnejad et al. 2008). Concisely, cells were harvested with 0.025% trypsin–EDTA, fixed with cold 70% ethanol, stained with 50 µg/ml PI solution containing 20 mg/ml RNase A and 0.1% Triton X-100, for 30 min and analyzed

with a FACScan cell sorter (Partec, Germany). The cell-cycle profiles were analyzed using Partec Flomax software.

Statistical Methods

All assays were repeated at least three times, and statistical significance was measured using one-way ANOVA and X^2 tests. P values of <0.05 were considered significant.

Results

BMSC Transfection

The cloned *NT-3* and *TrkC* cDNAs were received as a gift from professor Philip A. Barker in pDsRed1-N1 and pCMX vectors at Nhe I/BamH I and Hind III/Xba I cloning sites, respectively. Double digestion of the vectors yielded the predicted DNA sizes on agarose gel electrophoresis. The accuracy and integrity of cDNAs were further confirmed by polymerase chain reaction and DNA sequencing. The results of cotransfection revealed that the highest level of cotransfected cells was gained after using plasmid concentrations—from each of pDsRed1-N1-NT-3 and pCMX-TrkC plasmids—that were equal to the offered amount by the manufacturer company (data not shown).

Evaluation of Gene Expression

Relative gene expression analyses were assessed in genetically modified samples before and after neural induction. All reaction efficiencies were measured to be close to 100%.

Primary gene expression analysis revealed that in control sample, the gene expression profile was comparable to our previous results (Yaghoobi and Mowla 2006). While *NGF* and *BDNF* were gradually down-regulated, the expression of their receptors including *TrkA*, *TrkB* and even p75 neurotrophin receptor ($p75^{NTR}$)—the common death receptor—was increased. Neurofilament M (*NFM*) and microtubule-associated protein 2 (*MAP2*) revealed an increased pattern of gene expression subsequent to differentiation, compared to *NEUROD1* which showed a diminished one. The only difference was observed for *NT-3* which demonstrated a growing pattern by differentiation (Fig. 1a).

In *NT-3*/*TrkC*-cotransfected cells, the results were apparently changed. Almost all neurotrophins as well as their cognate receptors and $p75^{NTR}$ demonstrated an augmented pattern after differentiation. All neural markers including *NEUROD1* were also boosted. *NT-3* did not follow the over-expression pattern observed in control samples. An initial decrease and a sudden increase at 24 h were observed in these cells (Fig. 1b).

Secondary gene expression analysis indicated that by cotransfection of *NT-3*/*TrkC* vectors, the expression of *NGF*, *TrkA*, and *BDNF* was decreased, whereas *TrkB* was up-regulated before neural differentiation compared to the control (mock-transfected). *NT-3* and *TrkC* were both boosted significantly either before ($\sim$301 and $\sim$12.5 folds increase for *NT-3* and *TrkC*, respectively) or after ($\sim$50 and $\sim$270.6 folds increase for *NT-3* and *TrkC*, respectively) initiation of differentiation in cotransfected relative to mock-transfected cells. Although *MAP2* remained almost unaffected, the expression of *NEUROD1* and *NFM* was considerably enhanced in cotransfected compared with mock-transfected BMSCs after neural induction (Fig. 2).

Assessment of Cell Viability and Apoptosis

MTT assay indicated that neural differentiation caused a reduction in the number of viable cells in mock-transfected compared to transfected BMSCs which was demonstrated by one-way ANOVA test ($P < 0.05$). In addition, while having no significant impact on viability of the cells after pre-differentiation, cotransfection along with individual transfection of *NT-3* and *TrkC* remarkably recovered cell viability following neural differentiation (Fig. 3).

A significant reduction ($\sim$4 folds) in apoptosis rate of *NT-3*/*TrkC*-cotransfected BMSC-derived neural-like cells (2.94%) relative to mock-transfected differentiated cells (11.42%) was detected by means of Annexin-V-FLUOS staining apoptosis detection kit (Fig. 4).

The results also demonstrated a 24.3% rate of apoptosis in control transfected compared to *NT-3*/*TrkC*-cotransfected cells (6.8% amount of cell death) after flow cytometry of PI stained cells (Fig. 5).

Discussion

The success of cell therapy depends on high survival rate and synthetic potential of transplanted cells.

In fact, a major trait of BMSCs is their neuroprotection potential or the ability to secrete various growth, differentiation, and angiogenesis inducing proteins (Chen et al. 2005). Neurotrophin secretion is also considered as a major factor involved in trans-differentiation of BMSCs (Hokari et al. 2008). Another significant feature of BMSCs is that their genetic modification to improve their efficiency of function and survival is easily practical. As *NT-3* and *TrkC* expression could not be detected in our previous studies (Yaghoobi and Mowla 2006), we manipulated BMSCs to simultaneously over-express *NT-3* (as a growth factor) and its receptor *TrkC*. This approach integrated the two

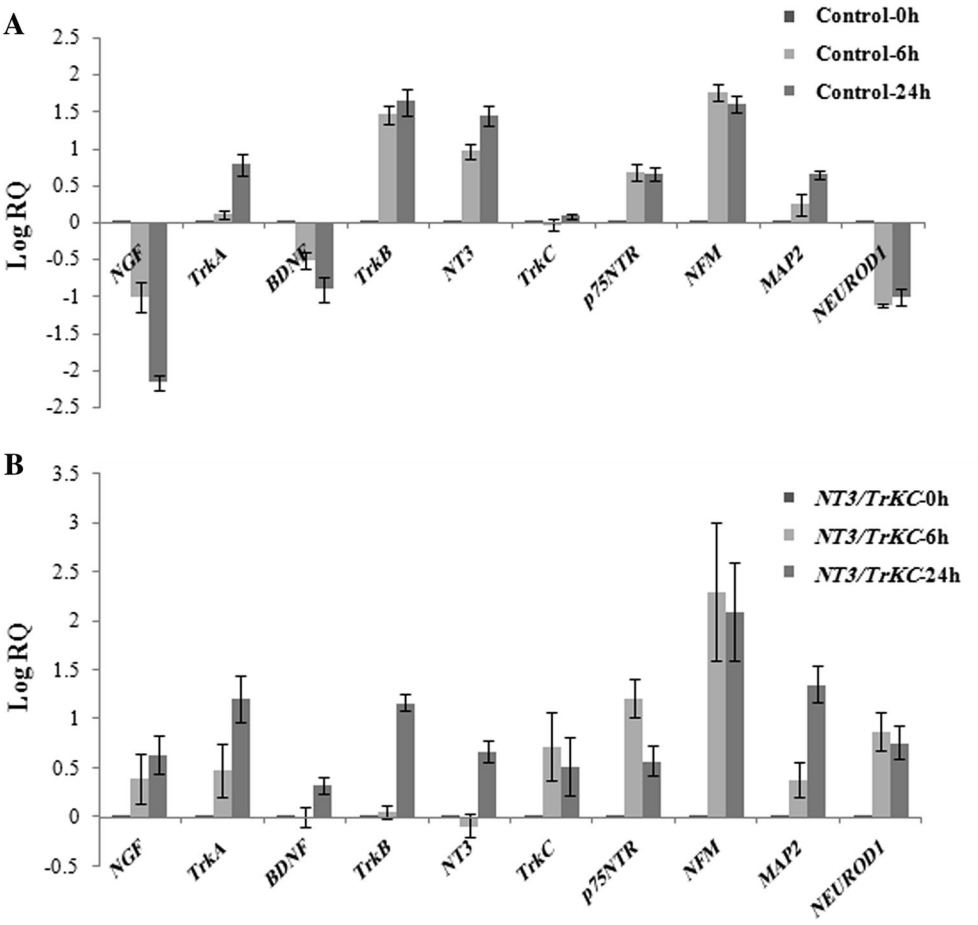


Fig. 1 The expression profile of neurotrophins, their receptors, and neural markers in mock (**a**) and *NT-3/TrkC* (**b**)—transfected BMSCs before and at 6 and 24 h following neural differentiation compared to the basal expression level in undifferentiated cells. **a** Except *NGF*, *BDNF*, and *NEUROD1* marker, other genes revealed an increased expression pattern after neural induction. **b** Almost all of the examined genes—including *NEUROD1*—showed an elevated level of expression following differentiation in *NT-3/TrkC*-cotransfected cells. For simplification, just the data for early (6 h) and late (24 h) stages of differentiation are illustrated

aforesaid characteristics of BMSCs, with the aim of fortifying the neuroprotection and differentiation efficiency of BMSCs in cell-therapy experiments through auto- and paracrine signaling as well as creating a route to deliver impermeable neurotrophins across blood brain barrier (Blesch 2006; Caplan 2007; Hokari et al. 2008).

The primary gene expression data for control sample were in accordance with our earlier studies except for *NT-3* that revealed an increased expression by differentiation (Fig. 1a) (Yaghoobi and Mowla 2006; Yaghoobi et al. 2005). The minor changes between the two studies may be as a consequence of difference in sample and/or the employed technique for detection. This study applied control plasmid-transfected cells instead of intact BMSCs and Real-Time qRT-PCR for detection which is more sensitive than traditional qRT-PCR. Although our previous data did not detect *NT-3* in BMSCs (Yaghoobi and Mowla 2006), some other investigations have stated *NT-3* expression beside the expression of other growth factors including *NGF*, *BDNF*, *GDNF*, *VEGF*, *CNTF*, *bFGF*, and *HGF* in these cells (Chen et al. 2005; Hokari et al. 2008). Although *NEUROD1* is considered to be required for survival and maturation of adult born neurons (Gao et al. 2009), its expression level was decreased following differentiation in control sample (Fig. 1a).

The primary gene expression data for *NT-3/TrkC*-cotransfected cells indicated that almost all the genes under study become over-expressed after differentiation. Unlike the control sample, a dramatic *TrkC* over-expression was observed following differentiation. $p75^{NTR}$ over-expression was also observed following differentiation in accordance with control (Fig. 1b). Previous reports have found that *NT-3* application leads to both *TrkC* and $p75^{NTR}$ over-expression in dorsal root ganglion(DRG) neurons (Verge et al. 1996). Therefore, besides increment as a direct result of *TrkC* transfection, an indirect enhancement resulted from *NT-3* over-expression may also be responsible for *TrkC* up-regulation. $p75^{NTR}$ overexpression may also be as a result of a kind of adaptation with *TrkC* over-expression to function as a co-receptor for *TrkC*.

Based on our secondary analysis of gene expression, a major increase in *NT-3* and *TrkC* was observed, specifically before and after neural differentiation for *NT-3* and *TrkC*, respectively. Alterations in *NT-3* and *TrkC* expression were somehow conversely related to the expression of other neurotrophins and *Trks*, correspondingly (Fig. 2).

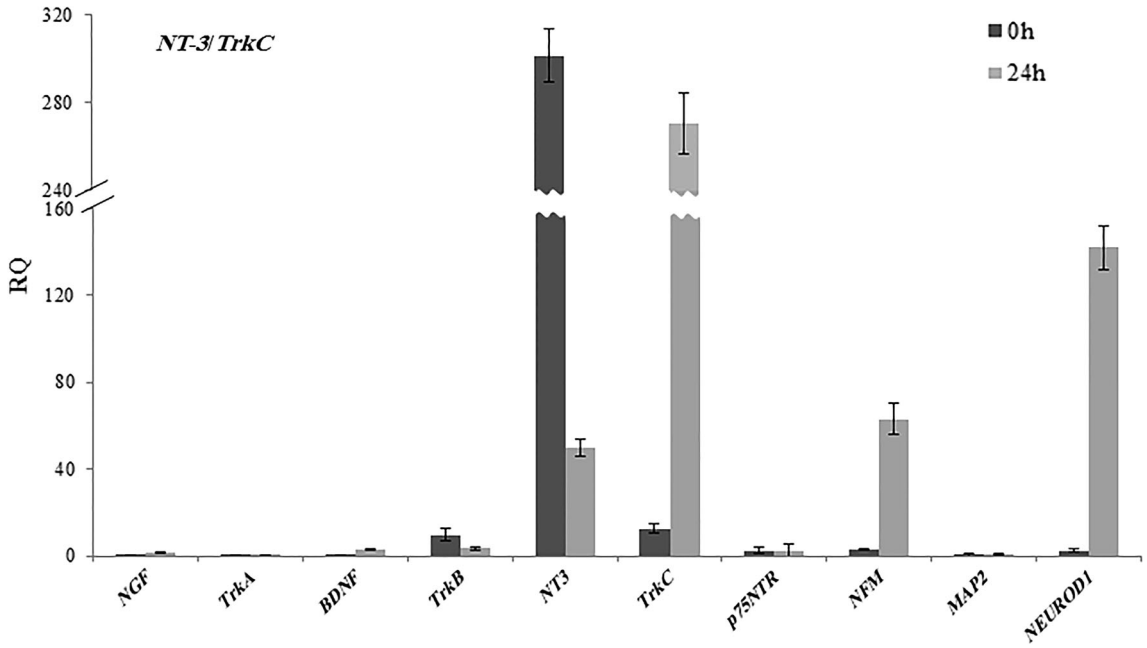


Fig. 2 Relative expression of neurotrophins, their receptors and neural markers in *NT-3*/*TrkC*-cotransfected BMSCs compared to mock at 0 and 24 h following neural differentiation. About 300 and 12 times of *NT-3* and *TrkC* augmentation, respectively, were observed before differentiation in cotransfected BMSCs corresponding to mock. At 24 h after differentiation, the *NT-3*/*TrkC*-cotransfected cells revealed 50, 270, 63, and 142 times increase in expression of *NT-3*, *TrkC*, *NFM*, and *NEUROD1*, respectively, in relation to the control cells. To facilitate analysis, the records for undifferentiated and 24 h differentiated *NT-3*/*TrkC*-cotransfected BMSCs are merely demonstrated

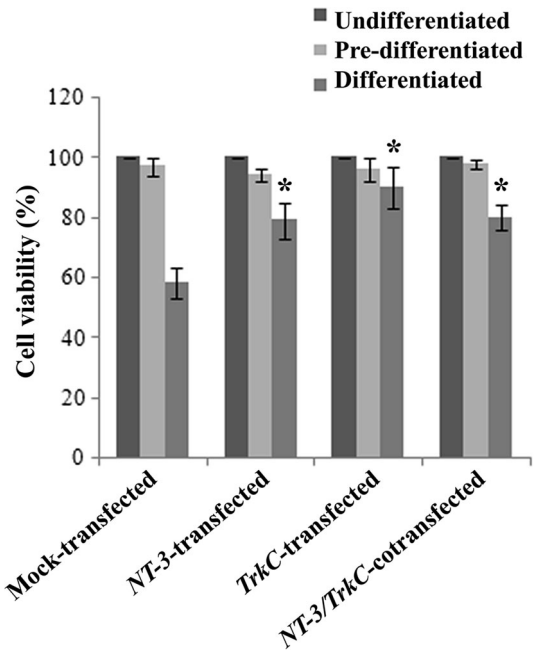


Fig. 3 MTT assay in mock-transfected, *NT-3*-transfected, *TrkC*-transfected, and *NT-3*/*TrkC*-cotransfected cells. Transfection of *NT-3* and/or *TrkC* plasmids had no significant impact on viability amount of bFGF-treated cells in pre-differentiated state related to the mock-transfected cells. But at about 6 h after neural induction, the amount of viable cells became considerably increased in *TrkC*-, *NT-3*-, and *NT-3*/*TrkC*-cotransfected in comparison with mock-transfected BMSCs

This entails that there may be an internal balance among the members of neurotrophin family. This hypothesis can even be generalized to *Trks*. Increased expression of neural markers (Fig. 2) following *NT-3*/*TrkC* cotransfection is also in accordance with previous reports. An in vitro study has demonstrated that a 7 day co-culture of *NT-3* expressing Schwann cells with *TrkC* expressing MSCs resulted in an increased neural differentiation in latter cells (Zhang et al. 2010). Another study has demonstrated the direct impact of adenoviral mediated *NT-3* over-expression on neural differentiation of MSCs (Zhang et al. 2006).

As a result of initial over-expression of survival factors including neurotrophins and *Trks* following differentiation, an increased survival and decreased apoptosis rates were observed in *NT-3*/*TrkC*-co-transfected cells despite $p75^{NTR}$ over-expression (Figs. 1, 2, 3). Actually, in the presence of all these factors for the sake of survival, $p75^{NTR}$ may function as a co-receptor for *Trks* that conduct the cell to survive rather than apoptosis (Farhadi et al. 2000; Hantzopoulos et al. 1994).

One of the main advantages of *NT-3* over-expression compared to other neurotrophins is its capacity to bind to all three kinds of *Trk* receptors (Ip et al. 1993). It has been shown that interaction of *NT-3* with *TrkA* and *TrkB* caused increased regeneration in various DRG neuronal subpopulations (Davies et al. 1995).

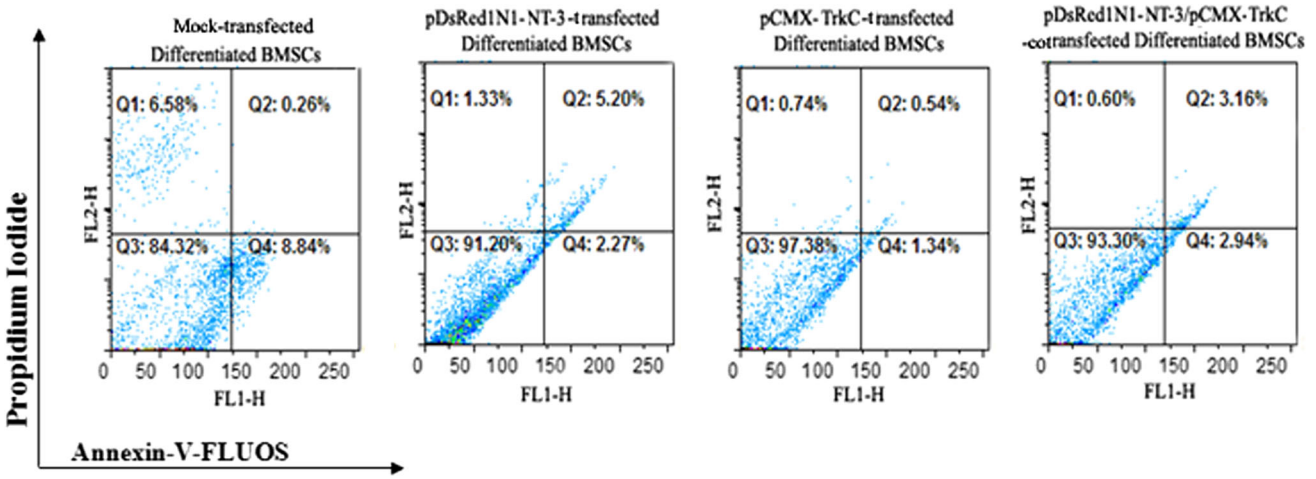
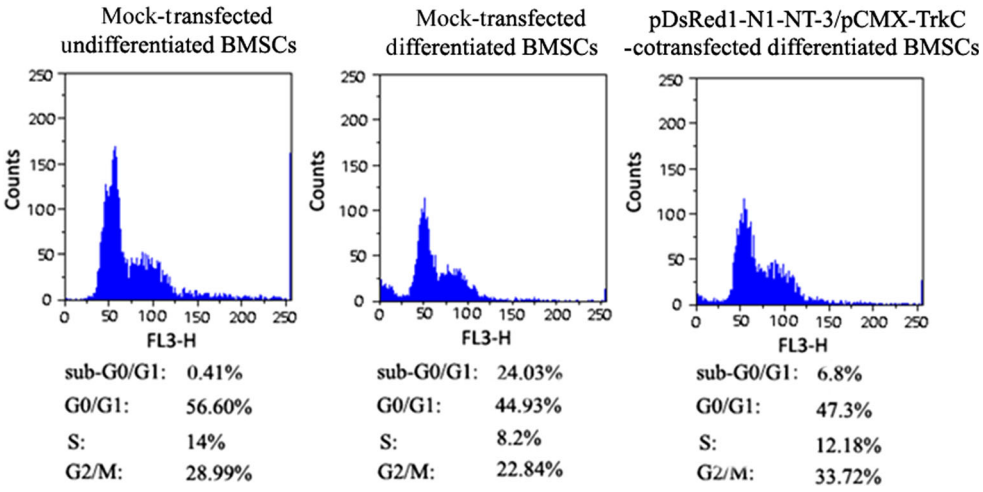


Fig. 4 Apoptosis assessment in mock-, *NT-3*-, and *TrkC*-transfected, and *NT-3*/*TrkC*-cotransfected cells after neural differentiation. The rate of apoptosis was significantly reduced in pDsRed1-N1-NT-3- and pCMX-TrkC-transfected (2.27 and 1.34%, respectively) and pDsRed1-N1-NT-3/pCMX-TrkC-cotransfected BMSCs (2.94%) compared to mock-transfected cells (11.42%), at about 6 h after neural induction

Fig. 5 Evaluation of apoptosis using cell-cycle examination. Apoptosis level was significantly lessened in pDsRed1-N1-NT-3/pCMX-TrkC-cotransfected cells (6.8%) related to mock BMSCs (24.03%), after differentiation



To the best of our knowledge, this is the first report studying the effects of simultaneous *NT-3*/*TrkC* over-expression in BMSCs. A former investigation has detected that coincident up-regulated *BDNF*/*TrkB* axotomized retina ganglionic cells can stay alive much more than the cells treated with *BDNF* alone (Cheng et al. 2002).

Taken together, our data revealed that simultaneous transfection of BMSCs with *NT-3* and its receptor, *TrkC*, resulted in increased survival and decreased apoptosis rate following neural differentiation. Furthermore, concomitant up-regulation of *NT-3* and *TrkC* led to increased expression of some neural markers. Consequently, these engineered cells may have successful transplantation consequences in neurologic disorders thanks to their increased potential in neural differentiation and survival. In addition, *NT-3* produced by BMSCs may have potential positive outcome in diseases in which *NT-3* undergoes a significant decrease (Blesch 2006).

Acknowledgements We are grateful to Dr. Phil Barker for kindly providing us the plasmids. This research was supported by a grant from Basij Elmi Organization (Grant No. 3657).

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Ethical Approval We certify that all applicable institutional regulations concerning the ethical use of animals were followed during the course of this research.

References

Blesch A (2006) Neurotrophic factors in neurodegeneration. Brain Pathol 16:295–303

Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 213:341–347

Chen Q et al (2005) Protective effects of bone marrow stromal cell transplantation in injured rodent brain: synthesis of neurotrophic factors. J Neurosci Res 80:611–619

Cheng L, Saphieha P, Kittlerova P, Hauswirth WW, Di Polo AT (2002) TrkB gene transfer protects retinal ganglion cells from axotomy-induced death in vivo. J Neurosci 22:3977–3986

Davies AM, Minichiello L, Klein R (1995) Developmental changes in NT-3 signalling via TrkA and TrkB in embryonic neurons. EMBO J 14:4482–4489

Edalat H, Hajebrahimi Z, Movahedin M, Tavallaei M, Amiri S, Mowla SJ (2011) p75NTR suppression in rat bone marrow stromal stem cells significantly reduced their rate of apoptosis during neural differentiation. Neurosci Lett 498:15–19

Edalat H, Hajebrahimi Z, Pirhajati V, Movahedin M, Tavallaei M, Soroush MR, Mowla SJ (2013) Transplanting p75-suppressed bone marrow stromal cells promotes functional behavior in a rat model of spinal cord injury. Iran Biomed J 17:140–145

Farhadi HF, Mowla SJ, Petrecca K, Morris SJ, Seidah NG, Murphy RA (2000) Neurotrophin-3 sorts to the constitutive secretory pathway of hippocampal neurons and is diverted to the regulated secretory pathway by coexpression with brain-derived neurotrophic factor. J Neurosci 20:4059–4068

Gao Z et al (2009) Neurodl is essential for the survival and maturation of adult-born neurons. Nat Neurosci 12:1090–1092

Hajebrahimi Z, Mowla SJ, Movahedin M, Tavallaei M (2008) Gene expression alterations of neurotrophins, their receptors and prohormone convertases in a rat model of spinal cord contusion. Neurosci Lett 441:261–266

Hantzopoulos PA, Suri C, Glass DJ, Goldfarb MP, Yancopoulos GD (1994) The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. Neuron 13:187–201

Hokari M, Kuroda S, Shichinohe H, Yano S, Hida K, Iwasaki Y (2008) Bone marrow stromal cells protect and repair damaged neurons through multiple mechanisms. J Neurosci Res 86:1024–1035

Ip NY et al (1993) Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. Neuron 10:137–149

Jafarnejad SM, Mowla SJ, Matin MM (2008) Knocking-down the expression of nucleostemin significantly decreases rate of proliferation of rat bone marrow stromal stem cells in an apparently p53-independent manner. Cell Prolif 41:28–35

Verge VM, Gratto KA, Karchewski LA, Richardson PM (1996) Neurotrophins and nerve injury in the adult. Philos Trans R Soc Lond B 351:423–430

Yaghoobi MM, Mowla SJ (2006) Differential gene expression pattern of neurotrophins and their receptors during neuronal differentiation of rat bone marrow stromal cells. Neurosci Lett 397:149–154

Yaghoobi MM, Mowla SJ, Tiraihi T (2005) Nucleostemin, a coordinator of self-renewal, is expressed in rat marrow stromal cells and turns off after induction of neural differentiation. Neurosci Lett 390:81–86

Zhang W, Zeng YS, Zhang XB, Wang JM, Zhang W, Chen SJ (2006) Combination of adenoviral vector-mediated neurotrophin-3 gene transfer and retinoic acid promotes adult bone marrow cells to differentiate into neuronal phenotypes. Neurosci Lett 408:98–103

Zhang YQ, Zeng X, He LM, Ding Y, Li Y, Zeng YS (2010) NT-3 gene modified Schwann cells promote TrkC gene modified mesenchymal stem cells to differentiate into neuron-like cells in vitro. Anat Sci Int 85:61–67