Research Article

Neurotrophic Features of Human Adipose Tissue-Derived Stromal Cells: In Vitro and In Vivo Studies

Wanda Lattanzi,1 Maria Concetta Geloso,1 Nathalie Saulnier,2 Stefano Giannetti,1 Maria Ausiliatrice Puglisi,2 Valentina Corvino,1 Antonio Gasbarrini,2 and Fabrizio Michetti1

1 Institute of Anatomy and Cell Biology, Università Cattolica del Sacro Cuore, 00168 Rome, Italy
2 Department of Internal Medicine and Gastroenterology, Università Cattolica del Sacro Cuore, 00168 Rome, Italy

Correspondence should be addressed to Maria Concetta Geloso, mc.geloso@rm.unicatt.it

Received 7 July 2011; Accepted 16 September 2011

Copyright © 2011 Wanda Lattanzi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Due to its abundance, easy retrieval, and plasticity characteristics, adipose-tissue-derived stromal cells (ATSCs) present unquestionable advantages over other adult-tissue-derived stem cells. Based on the in silico analysis of our previous data reporting the ATSC-specific expression profiles, the present study attempted to clarify and validate at the functional level the expression of the neurospecific genes expressed by ATSC both in vitro and in vivo. This allowed evidencing that ATSCs express neuro-specific trophins, metabolic genes, and neuroprotective molecules. They were in fact able to induce neurite outgrowth in vitro, along with tissue-specific commitment along the neural lineage and the expression of the TRKA neurotrophin receptor in vivo. Our observation adds useful information to recent evidence proposing these cells as a suitable tool for cell-based applications in neuroregenerative medicine.

1. Introduction

Adipose-tissue-derived adult pluripotent cells, commonly known as adipose tissue stromal cells (ATSCs) are mesenchymal stem cells (MSCs) residing in the connective stroma of adipose tissue. They represent a valuable source of adult stem cells, being easily isolated from an abundant and accessible tissue [1–3]. Their plasticity along with the ease of in vitro culturing and propagation makes them the most used cell type in a wide range of tissue regeneration applications [4–7].

We have previously shown the ATSC-specific molecular properties, by comparatively analyzing the geno-mewide expression profiles of MSCs from different adult tissues [3]. The study allowed indicating the main molecular features which regulate the stemness maintenance of MSCs and a more extensive plasticity of ATSC in vitro. The complete result dataset of this previous study (available at the Gene Expression Omnibus (GEO) database, http://www.ncbi.nlm.nih.gov/gds, accession number GSE8954) [3] also indicated that ATSCs specifically express neurospecific genes.

The purpose of this study is to extract the biologically significant genes from this dataset and validate the functional relevance of the neurotrophic genes expressed by ATSC both in vitro and in vivo.

2. Materials and Methods

2.1. In Silico Biological Analysis of the Microarray Dataset. In order to identify the candidate genes involved in the neurotrophic properties of ATSCs, the gene list of ATSC-specific genes obtained through the microarray-based gene profiling of ATSC compared to bone-marrow-derived mesenchymal cells (BMSCs) and fibroblasts (http://www.ncbi.nlm.nih.gov/gds, accession number GSE8954) [3] underwent an ad hoc biological analysis, aimed at finding neurologically relevant genes. For this purpose, the list of 441 genes specifically upregulated in ATSC (P value <0.01), resulting from the statistical analysis (see [3] for statistical methods used in data analysis), were categorized according to the “biological function” annotations implemented from the Gene Ontology.
Annotiation (GOA) database (http://www.ebi.ac.uk/GOA/). Specific neuroprotective, neurodevelopmental, and/or neurotrophic functions were further studied using the “Gene Reference Into Function” tool in GenBank (http://www.ncbi.nlm.nih.gov/gene/about-generif).

2.2. Patients and Specimens. Adipose tissue (AT) specimens were obtained by liposuction from healthy volunteers (mean age 40.2 ± 14.2 years) upon obtaining a written consent. A skin biopsy was obtained from the retroauricular region of a healthy male donor (aged 45) and served for the isolation of human dermal fibroblasts (HDF). Individuals data were handled confidentially and anonymously. All the procedures employed in this study were approved by the ethical committee of the Catholic University of Rome (Rome, Italy; number P552 (A.779)/CE2007).

2.3. Chemicals and Reagents. Cell culture media and supplements were purchased from Lonza (Basel, Switzerland). Enzymes, growth factors, and all other chemicals used in this study were purchased from Sigma (Sigma-Aldrich, St Louis, Mo, USA), unless otherwise specified.

2.4. ATSC Isolation and Culture. Mesenchymal stromal cells were isolated in primary culture from the liposaspirates, as already described elsewhere [3]. Briefly, AT was extensively washed, mechanically fractionated, and digested using 0.1% collagenase type VIII. The lysed tissue was then filtered through a 100 μm mesh, and the cell suspension was centrifuged. The cell pellet was then plated in T75 tissue culture flasks using Dulbecco’s Modified Eagle’s Medium (DMEM), supplemented with 10% FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.2 mg/mL fibroblast growth factor beta (bFGF). Cells were subcultured as previously described [3] and then used for in vitro and in vivo experiments, as detailed in the following paragraphs. ATSCs growth kinetics up to fifteen culture passages and their immunophenotype were assessed as already described elsewhere [6].

2.5. HDF Isolation and Culture. Dermal fibroblast were isolated in primary culture from the skin biopsy and cultured as previously described [8]. These cells served as a mesodermal-derived differentiated controls to produce the conditioned medium (HDF-CM) used in the in vitro experiments (see following paragraphs).

3. In Vitro Experimental Procedures: Neural Cell Line Cultures and Treatments

In order to assess the functional significance of the neurotrophic genes specifically expressed by ATSCs, LAN5 and PC12 cells were used as neural undifferentiated cell lines for the in vitro experiments. These cell lines are commonly employed as valuable models to study the neuronal differentiation and degeneration processes in vitro [9–13].

3.1. Cell Lines and Treatments. The human LAN-5 dopaminergic cells were grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 2 mM glutamine, 40 μg/mL gentamycin, and 10% heat-inactivated fetal calf serum (FCS), according to standard protocols [13]. Cells were plated at a 10^4/cm² seeding density in 24-well plates. The day after plating, ATSCs were seeded in the same wells using a 10^4/cm² seeding density. In addition, separate wells of LAN5 cells were cultured in presence of ATSC-conditioned medium (ATSC-CM), which was obtained by filtering through a 0.2 μm cellulose acetate filter the supernatant medium of subconfluent ATSC cultures. Thereafter, both LAN5-ATSC cocultures and ATSC-CM-treated cells were grown for three days without changing the culture medium.

The rat PC12 noradrenergic cell line was seeded at a density of 5000 cells/cm² in RPMI 1640 medium containing 5% fetal calf serum and 10% horse serum and grown till 80% confluence, according to standard protocols [12]. Between the third and the fourth culture passage, cells were plated in 24-well plates, using a 10^5/cm² seeding density. The day after plating, the PC12 culture medium was replaced by either ATSC-CM or HDF-CM. LAN5 and PC12 cells in standard culture medium served as controls in the experiments. As PC12 cells are known to differentiate along a functional neuronal phenotype upon NGF treatment, cells primed with 50 ng/mL of NGFβ were used as positive control of differentiation [9]. Cellular morphology was evaluated by an invertoscope up to four days of culture.

4. In Vivo Experimental Procedures: Neonatal Rat Brain ATSC Inoculation

4.1. Adenoviral-Mediated Cell Transduction. In order to make ATSC recognizable in living tissues, cells were transfected using a defective adenoviral vector carrying the enhanced green fluorescent protein (AdEGFP) as a reporter gene. AdEGFP stocks were kindly provided by the Vector Core Facility of the University of Pittsburgh (Pa, USA). Cells were plated at a 10^5/cm² seeding density and treated with AdEGFP using a multiplicity of infection (MOI) of 100 plaque-forming units (pfu)/cell. The efficiency of cell transduction was assessed observing fluorescent cells 48 hours later using an invertoscope equipped with a fluorescent lamp. EGFP-expressing cells were then inoculated in neonatal rats, as further described.

4.2. Cell Transplantation. Human ATSCs were transduced with Ad.eGFP 48 hours prior to in vivo transplantation. The surgery was performed on neonatal rats at postnatal day 1 (P1), after the induction of deep anesthesia by hypothermia. A small parietal hole was made into the skull above the frontal cortex, and cells were slowly injected into the lateral ventricle (1 mm posterior to the bregma, 1 mm lateral to the midline, and 2–2.5 mm ventral to the pial surface) using a glass micropipette coupled to a Hamilton microsyringe. For each animal treated, 5 x 10^4 ATSCs suspended in 1 μL of Puck’s saline A (Invitrogen, Carlsbad, Ca) were used. Sham-operated animals were injected with the same volume of...
saline solution. Following treatment, the skin was rapidly sutured, the pups were warmed under a lamp and returned to the dame. All animal protocols used have been approved by the Animal Experimentation Committee of the Catholic University of Rome.

4.3. Tissue Processing. The animals were sacrificed 7 and 15 days after injection \( (n = 6 \text{ for each group of ATSC treated rats, and } n = 3 \text{ for each group of sham-treated animals}) \). Under deep anaesthesia (ketamine/diazepam 1:1 i.p.), they were perfused through the aorta with 100 mL of saline solution, followed by 100 mL of 0.01 M, pH 7.4 PBS, and 4% paraformaldehyde. Thirty minutes after perfusion, the brains were removed from the skull, postfixed in 4% PBS paraformaldehyde for 2 h and immersed in 30% sucrose. Serial 40 \( \mu \)m thick coronal sections were cut on a freezing microtome. The first series of sections was mounted in Vectashield (Vector, UK) for fluorescent evaluation of eGFP-expressing cells. Other series of adjacent sections were processed for immunohistochemistry.

4.4. Immunohistochemistry. Anti-GFAP (polyclonal, Dako, Glostrup, Denmark, 1:1000 overnight at 4°C), -Doublecortin (polyclonal, Chemicon, Temecula, Ca, 1:3000, overnight at 4°C), -NeuN (monoclonal, Chemicon, Temecula, Ca, 1:500, 48 h at 4°C), -O4 (monoclonal, Chemicon, Temecula, Ca, 1:500, overnight at 4°C), and -TrKA (Santa Cruz Biotechnology, Heidelberg, Germany, 1:1000 overnight at 4°C) were revealed using cyanine fluorochromes-labeled secondary antibodies (donkey anti-mouse Cy3 or donkey anti-rabbit Cy3, Jackson Immunoresearch Laboratories, West Grove, Pa, 1:400) following incubation for 1 hour at RT. Sections were mounted in Vectashield for fluorescent visualisation of labeled cells. Controls were prepared by omitting the primary antibodies.

The colocalization of eGFP with the above-mentioned markers was examined with a Zeiss LSM 510 confocal laser scanning microscopy system.

5. Results

5.1. ATSCs Express Neurospecific Genes. Data extracted from previously published microarray data showed the selective upregulation of 441 genes \( (P < 0.01) \) in ATSC compared to BMSC and human fibroblast MRC5 cells (Figure 1). The in silico biological analysis of the microarray data (GEO dataset number GSE8954) allowed to identify a short list of biologically relevant genes, involved in neuroprotection, neural developmental processes, and neurotrophic functions (see Table 1). In particular, this 12-transcript list included genes, namely, nerve growth factor beta (NGFB), neuropilin 1
Figure 2: *In vitro* neurotrophic effects of ATSC. LAN-5 human neuroblasts and PC12 rat cells were cultured either ATSC-conditioned medium or co-cultured with ATSC and morphological modifications were monitored over time: (a) LAN5 in standard culture medium; (b) LAN5 cultured in ATSC-CM for 72 hours; (c) and (d) LAN-5 co-cultured with ATSC using a cell density of $10^4$ cell/cm² for both cell populations; (e) PC12 in standard culture medium; (f) PC12 cultured in ATSC-CM for 4 days; (g) PC12 cultured in βNGF 100 ng/mL for 4 days; (h) PC12 cultured in HDF-conditioned medium for 4 days. Arrows show evidence of neurite outgrowth; asterisk (*) indicate ATSC in culture. Scale bar 100 μm in all panels except for panel d = 10 μm.

Figure 3: Efficient adenoviral-mediated transduction of ATSC. ATSCs were transfected with 100 pfu/cell of AdEGFP and fluorescent cells were observed after 48 hours: nearly 80% cells were EGFP-positive as shown in the figure.

(NRP1), and GTP cyclohydrolase 1 (GCH1), encoding soluble neurotrophins which are known to mediate neuronal growth, differentiation, migration, and neuroprotection [9, 14, 15]. The neuronal cadherin CDH2 belongs to the major transmembranar signalling complex cadherin/catenin that plays a key role in neuronal processes during early development. It is activated during neural circuit formation and maturation to mediate axonal outgrowth and arborisation [16, 17]. Moreover, nearly all genes in the list are implicated in developmental processes within the nervous system, such as neurogenesis, neuron differentiation, axonogenesis, axon guidance, nerve growth, and glia differentiation and migration (Table 1). The phosphoribosyl pyrophosphate synthetase 1 (PRPS1) and the phosphoglycerate mutase 1 (PGAM1) genes are implicated in metabolic pathways which are essential in neuronal function and maintenance (see function details and references in Table 1).

5.2. ATSCs Induce Neurite Outgrowth in PC12 and LAN5 Cells. In order to evaluate the effects of the supposed neurotrophic properties of ATSC, the capability of inducing visible changes in cell morphology of neural cells was first assessed *in vitro*. For this purpose, LAN5 cells were either cultured in ATSC-CM or cocultured with human ATSC for three days. Both cells cultured in ATSC-CM (Figure 2(b)) and those in coculture (Figures 2(c)-2(d)) displayed evident changes in shape and morphology, compared to those grown in standard culture medium (Figure 2(a)). The morphological changes consisted in the formation and elongation of neurite-like processes observed in discrete loci of the culture plate. The outgrown neurites seemed to establish contacts with both neural cells and ATSC in culture (Figure 2(d)).

In addition, the adrenergic PC12 cell line was cultured in presence of ATSC-conditioned medium (ATSC-CM) for four days. PC12 primed with βNGF and PC12 cultured in HDF-CM was used as positive and negative neuro-differentiation controls, respectively. The morphological analysis showed the extensive outgrowth and extension of neurite-like structures in both βNGF- and ATSC-CM-treated cells exhibiting essentially overlapping features (Figure 2(f)-2(g)), compared to cells cultured in standard medium (Figure 2(e)). Cells grown in HDF-CM showed clear morphological signs of distress, becoming small-rounded vacuolized cells, with a marked tendency to detach (Figure 2(h)).
Table 1: Selected ATSC-specific upregulated genes involved in neurospecific functions ($P < 0.01$).

| Gene symbol | Gene bank | Gene name                                      | Neurospecific functions | Process                                             | References |
|-------------|-----------|-----------------------------------------------|-------------------------|----------------------------------------------------|------------|
| SLC1A1      | NM_004170.5 | Solute carrier family 1, member 1             | protection              | Protection against glutamate neurotoxicity         | [18]       |
| CDH2        | NM_001792.3 | Cadherin 2, type 1, N-cadherin (neuronal)     | development             | Pre-to-postsynaptic adhesion neuronal migration     | [19]       |
| CELF2       | NM_001025077.2 | CUG triplet repeat, RNA binding protein 2 | trophism                | Axonogenesis synapse assembly                       |            |
| VLDLR       | NM_003383.3 | Very low density lipoprotein receptor         | metabolism              | Motor neuron survival splicing control during       | [20]       |
| NRP1        | NM_003873  | Neuropilin 1                                  |                         | Nervous system development lipid uptake in neurons  |            |
| NGFB        | NM_002506.2 | Nerve growth factor, beta polypeptide         |                         | and astrocytes                                      |            |
| ENC1        | NM_003633.2 | Ectodermal-neural cortex                      |                         | Cell survival axon guidance                        | [15]       |
| GCH1        | NM_000161.2 | GTP cyclohydrolase 1                          |                         | Migration and invasion                              |            |
| FGF2        | NG_012449.1 | Fibroblast growth factor 2                    |                         | Neuron differentiation nerve growth                 | [9]        |
| NDN         | NM_002487.2 | Necdin homolog (mouse)                       |                         | Anti-apoptotic nervous system development          | [21]       |
| PRPS1       | NM_002764.3 | Phosphoribosyl pyrophosphate synthetase 1     |                         | Protection from brain damaging events secreted by   | [14]       |
| PGAM1       | NM_002629.2 | Phosphoglycerate mutase 1 (brain)            |                         | astrocyte                                           |            |

5.3. In Vivo Analysis of ATSC-Specific Neurotrophic Features.
The functional significance of the ATSC-specific upregulation of genes involved in the neural lineage has been further investigated in vivo after transplantation of ATSCs in the neonatal rat brain. ATSCs were efficiently transduced with Ad.eGFP prior to in vivo transplantation (Figure 3).

Histological examination of ATSC-transplanted young rats sacrificed 7 days after transplantation showed clusters of eGFP-positive ATSCs, characterized by rounded morphology, localized in the wall of the lateral ventricle, near the needle tract, surrounded by GFAP positive astroglial endings (Figure 4(a) A–C). In particular, based on the results
Figure 4: Engraftment of human ATSCs within newborn rat brain. (a) Confocal microscopy micrographs showing the engraftment of eGFP-positive (green; A, D) ATSCs within newborn rat brain 1 week after cell infusion. ATSCs exhibit a round morphology (A, C), are surrounded by GFAP-positive astrocytes (red; B, D), and express TRKA (red, B, D, arrows). (b) Engraftment and in vivo differentiation of human ATSCs within newborn rat hippocampus 2 weeks after implantation. Confocal images of GFAP (red; A) or TRKA (red; D) immunolabeled eGFP (green; B, E) expressing ATSCs. Engrafted cells express the astrocytic marker GFAP (yellow, C) and the TRKA receptor (yellow, F). Scale bars: (a) A–C 120 μm, (a) D–F 420 μm, (b) A–C 80 μm, and (b) D–F 60 μm.
observed in vitro, we assessed the expression of the anti-NGF-β receptor, as to further investigate the significance of the NGF/TRKA signaling pathway. ATSCs exhibited immunopositivity for the TRKA antibody 7 days after transplantation (Figure 4(a) D–F). At this time point, no colocalization with neuronal (Doublecortin, NeuN), astroglial (GFAP), or oligodendroglial (O4) markers were observed (not shown).

Histological examination of young rats sacrificed 15 days after transplantation confirmed the survival of ATSCs in the brain of injected animals. Grafted cells examined at this time point were mainly localized within the brain parenchyma, near the ventricular system and frequently in the hippocampus. They showed a bipolar or multipolar morphology with processes extending in various directions. Interestingly, confocal microscopy examination revealed that many of these eGFPP- positive ATSCs coexpressed also the astroglial marker GFAP (Figure 4(b) A–C), while no colocalization between eGFAP and Doublecortin, NeuN, or O4 was found (not shown). Virtually all engrafted ATSCs expressed immunopositivity for anti-TRKA antibody (Figure 4(b) D–F). Sham-operated animals exhibited only a mild GFAP-stained glial reaction around the needle tract (not shown).

6. Discussion

Different evidences indicated that transplanted MSCs promote enogenous repair of neurologically damaged areas and neural differentiation, via the release of soluble trophic factors and cytokines [27].

In particular, recent studies indicated that ATSC culture medium should contain neurotrophic factors, which were able to induce neuritogenesis in PC12 cells in vitro and protect brain from both hypoxic damage and glutamate neurotoxicity [28–30]. Nonetheless, only selected molecules have been dosed in ATSCs as possible neurotrophic candidates [28–31], while the expression of a wider panel of neuro-specific molecules has not been assessed in ATSCs so far.

The possible complete list of neurotrophic/neuroprotective factors specifically expressed by ATSC is proposed in this study, as a result of the in silico analysis of differentially expressed genes in MSC isolated from different adult tissues [3]. This revealed that ATSCs strongly and specifically express at least three neurotrophins: NGFB, NRP1, and FGF2. These secreted molecules reasonably represent the molecular background of ATSC-neurotrophic features. The in vitro assays in this study demonstrated that ATSCs could in fact induce neurite outgrowth not only in PC12, but also in human neuroblasts (LAN5 cell line). The induction of neuronal differentiation should be the result of the demonstrated presence of soluble secreted factors in ATSC culture medium [28] along with cell-to-cell contacts with neural cells in vitro. Thus, this event could be reasonably mediated by both NGFB, which promotes neuronal differentiation [9], and NRP1 that guides axon growth [15]. Also the nonneurospecific growth factor FGF2 could play a role in this event, being able to promote neurogenesis [22]. In addition, the adhesion molecule CDH2 that is expressed on the plasma membrane and is involved in axonogenesis and synapse assembly [19] could play a role in ATSC-mediated neuronal differentiation of LAN-5 cells. Although, the possibility that other factors participate in mediating this effects cannot be excluded.

Our data could also suggest that ATSCs neurotrophic function resides in a sort of astrocyte-like phenotype, as they specifically express genes belonging to the glial phenotype, including VLDR, FGF2, and NDN, according to GOA annotations. To this end, the neccin homolog (NDN) gene, involved in the NGFB signalling pathway, is particularly relevant, as it drives glial migration during nervous system development and is expressed in the cell projections [23]. Although the neural transdifferentiation capacity of MSCs has been largely debated, many recent studies emphasise the possibility of both bone marrow- and adipose tissue derived-undifferentiated stromal cells to differentiate along the neuroectodermal lineage to neuronal-like cells of the ectodermal lineage, mainly in vitro [32–42].

Recent data indeed assess the importance of cell-cell interactions along with the release of growth factors from the host tissue in ATSCs neural transdifferentiation [43]. In line with these observations, the results obtained in vivo, following cell implantation in the neonatal rat brain, indicate that ATSCs survive, migrate, and essentially differentiate toward an astroglial fate. Taken together, our observations suggest that ATSCs show a predisposition to the neural fate as they express a molecular phenotype resembling neural commitment in vitro and transdifferentiate along the neural lineage in vivo.

Recent reports evidence the successful implantation and migration of ATSCs in vivo using experimental models of rat brain ischemia, where they were able to promote functional recovery [44–47]. In addition, different groups reported the neural transdifferentiation of ATSCs transplanted in the injured spinal cord [48, 49], evidencing that, when detached from the physiological niche, they express ectodermal neural markers [50]. We may speculate that secreted soluble factors from neighbouring cells and physical reciprocal contacts with neural cells may cause/facilitate transdifferentiation processes, as also indicated by the expression of the NGF receptor TRKA by transplanted ATSCs. This evidence, reported in in vitro studies [51], could suggest a possible autocrine mechanism on ATSC, as they express NGFB in vitro, although the functional significance of this observation deserves further studies.

Taken together, the results obtained in this study seemed to indicate that ATSC neurotrophic features reside in their specific capability of expressing not only secreted neurotrophins/neuroprotective molecules, but also structural protein-coding genes, mimicking the astrocyte function in sustaining neurons metabolism and function in the central nervous system and being able to differentiate into astrocytes. These properties, along with their reported capacity to migrate in injured tissues, could suggest possible future applications of ATSCs in many diverse neurological contexts.
Author Contribution

Wanda Lattanzi and Maria Concetta Geloso contributed equally to this work.

References

[1] P. A. Zuk, M. Zhu, P. Ashjian et al., “Human adipose tissue is a source of multipotent stem cells,” Molecular Biology of the Cell, vol. 13, no. 12, pp. 4279–4295, 2002.
[2] A. J. Katz, A. Tholpady, S. S. Tholpady, H. Shang, and R. C. Ogle, “Cell surface and transcriptional characterization of human adipose-derived adherent stromal (HADAS) cells,” Stem Cells, vol. 23, no. 3, pp. 412–423, 2005.
[3] N. Saulnier, M. A. Puglisi, W. Lattanzi et al., “Gene profiling of bone marrow- and adipose tissue-derived stromal cells: a key role of Kruppel-like factor 4 in cell fate regulation,” Cytotherapy, vol. 13, no. 3, pp. 329–340, 2011.
[4] P. A. Zuk, M. Zhu, H. Mizuno et al., “Multilineage cells from human adipose tissue: implications for cell-based therapies,” Tissue Engineering, vol. 7, no. 2, pp. 211–228, 2001.
[5] F. Guilak, K. E. Lott, H. A. Awad et al., “Clonal analysis of the differentiation potential of human adipose-derived adult stem cells,” Journal of Cellular Physiology, vol. 206, no. 1, pp. 229–237, 2006.
[6] N. Saulnier, W. Lattanzi, M. A. Puglisi et al., “Mesenchymal stromal cells multipotency and plasticity: induction toward the hepatic lineage,” European Review for Medical and Pharmacological Sciences, vol. 13, supplement 1, pp. 71–78, 2009.
[7] C. Parrilla, W. Lattanzi, A. R. Fetoni, F. Bussu, E. Pola, and G. Paludetti, “Ex vivo gene therapy using autologous dermal fibroblasts expressing HlMP3 for rat mandibular bone regeneration,” Head and Neck, vol. 32, no. 3, pp. 310–318, 2010.
[8] W. Lattanzi, C. Parrilla, A. Fetoni et al., “Ex vivo-transduced autologous skin fibroblasts expressing human Lim mineralization protein-3 efficiently form new bone in animal models,” Gene Therapy, vol. 15, no. 19, pp. 1330–1343, 2008.
[9] R. Tabakman, H. Jiang, I. Shahar, H. Arien-Zakay, R. A. Levine, and P. Lazarovic, “Neuroprotection by NGF in the PC12 in vitro OGD model: involvement of mitogen-activated protein kinases and gene expression,” Annals of the New York Academy of Sciences, vol. 1053, pp. 84–96, 2005.
[10] Z. Xu, D. Cawthon, K. A. McCastlain et al., “Selective alterations of transcription factors in MPP+-induced neurotoxicity in PC12 cells,” NeuroToxicology, vol. 26, no. 4, pp. 729–737, 2005.
[11] R. Businaro, S. Leone, C. Fabrizi et al., “S100B protects LAN-5 neuroblastoma cells against Aβ amyloid-induced neurotoxicity via RAGE engagement at low doses but increases Aβ amyloid neurotoxicity at high doses,” Journal of Neuroscience Research, vol. 83, no. 5, pp. 897–906, 2006.
[12] W. Lattanzi, C. Bernardini, C. Gangitano, and F. Michetti, “Hypoxia-like transcriptional activation in TMT-induced degeneration: microarray expression analysis on PC12 cells,” Journal of Neurochemistry, vol. 100, no. 6, pp. 1688–1702, 2007.
[13] C. Bernardini, W. Lattanzi, R. Businaro et al., “Transcriptional effects of S100B on neuroblastoma cells: perturbation of cholesterol homeostasis and interference on the cell cycle,” Gene Expression, vol. 14, no. 6, pp. 345–359, 2010.
[14] A. Chiarini, U. Armato, R. Pacchiana, and I. D. Pra, “Proteomic analysis of GTP cyclohydrolase 1 multiprotein complexes in cultured normal adult human astrocytes under both basal and cytokine-activated conditions,” Proteomics, vol. 9, no. 7, pp. 1850–1860, 2009.
[15] Q. Schwarz and C. Ruhrberg, “Neuregulin, you gotta let me know: should I stay or should I go?” Cell Adhesion and Migration, vol. 4, no. 1, pp. 61–66, 2010.
[16] D. L. Benson and H. Tanaka, “N-cadherin redistribution during synaptogenesis in hippocampal neurons,” Journal of Neuroscience, vol. 18, no. 17, pp. 6892–6904, 1998.
[17] M. Kadowaki, S. Nakamura, O. Machon, S. Krauss, G. L. Radice, and M. Takeichi, “N-cadherin mediates cortical organization in the mouse brain,” Developmental Biology, vol. 304, no. 1, pp. 22–33, 2007.
[18] A. Nicoulon, B. Canolle, F. Masmejean, B. Guillet, P. Pisano, and S. Lorret, “The neuronal excitatory amino acid transporter EAAC1/EAT3: does it represent a major actor at the brain excitatory synapse?” Journal of Neurochemistry, vol. 98, no. 4, pp. 1007–1018, 2006.
[19] J. Arikkath, “N-cadherin: stabilizing synapses,” Journal of Cell Biology, vol. 189, no. 3, pp. 397–398, 2010.
[20] K. Sakai, O. Tiebel, M. C. Ljunghberg et al., “A neuronal VLDLR variant lacking the third complement-type repeat exhibits high capacity binding of apoE containing lipoproteins,” Brain Research, vol. 1276, no. C, pp. 11–21, 2009.
[21] T. A. Kim, J. Lim, S. Ota et al., “NRP/B, a novel nuclear matrix protein, associates with p110(RB) and is involved in neuronal differentiation,” Journal of Cell Biology, vol. 141, no. 3, pp. 553–566, 1998.
[22] G. Mudo, A. Bonomo, V. Di Liberto, M. Frinchi, K. Fuxe, and N. Belluardo, “The FGF-2/FGFRs neurotrophic system promotes neurogenesis in the adult brain,” Journal of Neuro Transmitter, vol. 116, no. 8, pp. 995–1005, 2009.
[23] C. A. Ingraham, L. Wertalik, and N. F. Schor, “Necdin and neurotrophin receptors: interactors of relevance for neuronal resistance to oxidant stress,” Pediatric Research, vol. 69, no. 4, pp. 279–284, 2011.
[24] A. P. M. de Brouwer, H. van Bokhoven, S. B. Nabuurs, W. F. Arts, J. Christodoulou, and J. Duley, “PRPS1 mutations: four distinct syndromes and potential treatment,” American Journal of Human Genetics, vol. 86, no. 4, pp. 506–518, 2010.
[25] H. J. Kim, K. M. Sohn, M. E. Shy et al., “Mutations in PRPS1, which encodes the phosphoribosyl pyrophosphate synthetase enzyme critical for nucleotide biosynthesis, cause hereditary peripheral neuropathy with hearing loss and optic neuropathy (CMTX5),” American Journal of Human Genetics, vol. 81, no. 3, pp. 552–558, 2007.
[26] Y. Miyamae, I. Han, K. Sasaki, M. Terakawa, H. Isoda, and H. Shigemori, “3,4,5-tri-O-cafeoylquinic acid inhibits amyloid β-mediated cellular toxicity on SH-SY5Y cells through the upregulation of PGAM1 and G3PDH,” Cytotechnology, vol. 63, no. 2, pp. 191–200, 2011.
[27] S. A. Hardy, D. J. Maltman, and S. A. Przyborski, “Mesenchymal stem cells as mediators of neural differentiation,” Current Stem Cell Research and Therapy, vol. 3, no. 1, pp. 43–52, 2008.
[28] W. Xing, D. Zhimei, Z. Liming et al., “IFATS collection: the proteomic analysis of GTP cyclohydrolase 1 multiprotein complexes in cultured normal adult human astrocytes under both basal and cytokine-activated conditions,” Proteomics, vol. 9, no. 7, pp. 1850–1860, 2009.
[30] B. Tan, Z. Luan, X. Wei et al., “AMP-activated kinase mediates adipose stem cell-stimulated neuritogenesis of PC12 cells,” *Neuroscience*, vol. 181, pp. 40–47, 2011.

[31] D. X. Qian, H. T. Zhang, X. Ma, X. D. Jiang, and R. X. Xu, “Comparison of the efficiencies of three neural induction protocols in human adipose stromal cells,” *Neurochemical Research*, vol. 35, no. 4, pp. 572–579, 2010.

[32] J. Sanchez-Ramos, S. Song, F. Cardozo-Pelaez et al., “Adult bone marrow stromal cells differentiate into neural cells in vitro,” *Experimental Neurology*, vol. 164, no. 2, pp. 247–256, 2000.

[33] N. Bertani, P. Malatesta, G. Volpi, P. Sonego, and R. Perris, “Neurogenic potential of human mesenchymal stem cells revisited: analysis by immunostaining, time-lapse video and microarray,” *Journal of Cell Science*, vol. 118, no. 17, pp. 3925–3936, 2005.

[34] K. Mareschi, M. Novara, D. Rustichelli et al., “Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K+ channel types,” *Experimental Hematology*, vol. 34, no. 11, pp. 1563–1572, 2006.

[35] A. Cardozo, M. Ielpi, D. Gómez, and P. Argibay, “Differential expression of Shh and BMP signaling in the potential conversion of human adipose tissue stem cells into neuron-like cells in vitro,” *Gene Expression*, vol. 14, no. 6, pp. 307–319, 2010.

[36] S. Jang, H. H. Cho, Y. B. Cho, J. S. Park, and H. S. Jeong, “Functional neural differentiation of human adipose tissue-derived stem cells using bFGF and forskolin,” *BMC Cell Biology*, vol. 11, article 25, 2010.

[37] G. Lepski, C. E. Jannes, B. Strauss, S. K. N. Marie, and G. Nikkhah, “Survival and neuronal differentiation of mesenchymal stem cells transplanted into the rodent brain are dependent upon microenvironment,” *Tissue Engineering—Part A*, vol. 16, no. 9, pp. 2769–2782, 2010.

[38] P. A. Zuk, “The adipose-derived stem cell: looking back and looking ahead,” *Molecular Biology of the Cell*, vol. 21, no. 11, pp. 1783–1787, 2010.

[39] T. Kondo, A. J. Matsuoka, A. Shimomura et al., “Wnt signaling promotes neuronal differentiation from mesenchymal stem cells through activation of Tlx3,” *Stem Cells*, vol. 29, no. 5, pp. 836–846, 2011.

[40] Y. L. Yu, R. H. Chou, L. T. Chen et al., “EZH2 regulates neuronal differentiation of mesenchymal stem cells through PIP5K1C-dependent calcium signaling,” *Journal of Biological Chemistry*, vol. 286, no. 11, pp. 9657–9667, 2011.

[41] E. Garbayo, A. P. Raval, K. M. Curtis et al., “Neuroprotective properties of marrow-isolated adult multilineage-inducible cells in rat hippocampus following global cerebral ischemia are enhanced when complexed to biomimetic microcarriers,” *Journal of Neurochemistry*, vol. 119, no. 5, pp. 972–988, 2011.

[42] J. Y. Lim, S. I. Park, S. M. Kim et al., “Neural differentiation of brain-derived neurotrophic factor-expressing human umbilical cord blood-derived mesenchymal stem cells in culture via TkkB-mediated ERK and β-catenin phosphorylation and following transplantation into the developing brain,” *Cell Transplantation*, In press.

[43] D. Y. Wang, S. C. Wu, S. P. Lin, S. H. Hsiao, T. W. Chung, and Y. Y. Huang, “Evaluation of transdifferentiation from mesenchymal stem cells to neuron-like cells using microfluidic patterned co-culture system,” *Biomedical Microdevices*, vol. 13, no. 3, pp. 517–526, 2011.

[44] S. K. Kang, D. H. Lee, Y. C. Bae, H. K. Kim, S. Y. Baik, and J. S. Jung, “Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats,” *Experimental Neurology*, vol. 183, no. 2, pp. 355–366, 2003.

[45] S. T. Lee, K. Chu, K. H. Jung et al., “Slowed progression in models of Huntington disease by adipose stem cell transplantation,” *Annals of Neurology*, vol. 66, no. 5, pp. 671–681, 2009.

[46] S. Leu, Y. C. Lin, C. M. Yuen et al., “Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats,” *Journal of Translational Medicine*, vol. 8, article no. 63, 2010.

[47] Y. Ikegame, K. Yamashita, S. I. Hayashi et al., “Comparison of mesenchymal stem cells from adipose tissue and bone marrow for ischemic stroke therapy,” *Cytotherapy*, vol. 13, no. 6, pp. 675–685, 2011.

[48] H. T. Zhang, J. Luo, L. S. Sui et al., “Effects of differentiated versus undifferentiated adipose tissue-derived stromal cell grafts on functional recovery after spinal cord contusion,” *Cellular and Molecular Neurobiology*, vol. 29, no. 8, pp. 1283–1292, 2009.

[49] D. Arboleda, S. Forostyak, P. Jendelova et al., “Transplantation of predifferentiated adipose-derived stromal cells for the treatment of spinal cord injury,” *Cellular and Molecular Neurobiology*, vol. 31, no. 7, pp. 1113–1122, 2011.

[50] G. C. Kopen, D. J. Prockop, and D. G. Phinney, “Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 19, pp. 10711–10716, 1999.

[51] S. Dhar, E. S. Yoon, S. Kachgal, and G. R. D. Evans, “Long-term maintenance of neurally differentiated human adipose tissue-derived stem cells,” *Tissue Engineering*, vol. 13, no. 11, pp. 2625–2632, 2007.