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Transcriptional Characterization of Wnt and Notch Signaling Pathways in Neuronal Differentiation of Human Adipose Tissue-Derived Stem Cells

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Abstract Since the nervous system has limited self-repair capability, a great interest in using stem cells is generated to repair it. The adipose tissue is an abundant source of stem cells and previous reports have shown the differentiation of them in neuron-like cells when cultures are enriched with growth factors involved in neurogenesis. Regarding this, it could be thought that a functional parallelism between neurogenesis and neuronal differentiation of human adipose stem cells (hASCs) exists. For this reason, we investigated the putative involvement of Notch and Wnt pathways in neuronal differentiation of hASCs through real-time PCR. We found that both Wnt and Notch signaling are present in proliferating hASCs and that both cascades are downregulated when cells are differentiated to a neuronal phenotype. These results are in concordance with previous works where it was found that both pathways are involved in the maintenance of the proliferative state of stem cells, probably through inhibition of the expression of cell-fate-specific genes. These results could support the notion that hASCs differentiation into neuron-like cells represents a regulated process analogous to what occurs during neuronal differentiation of NSCs and could partially contribute to elucidate the molecular mechanisms involved in neuronal differentiation of adult human nonneural tissues.

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Introduction

Neurodegenerative diseases are difficult to treat because damaged neurons have a limited capacity for regeneration and repair. Therefore, efforts to identify suitable tissue sources to replace lost neuronal populations have intensified (Reynolds and Weiss 1992; Richards et al. 1992). Stem cells are an exciting source because they can undergo expansion and neuronal differentiation in vitro and in vivo (Bain et al. 1995; McKay, 1997; Gage, 2000; Freed et al. 2001). Adipose tissue is an abundant source of stem cells that can be easily isolated (Hauner et al. 1987; Gronthos et al. 2001), termed adipose tissue-derived stem cells (ASCs) (Zuk et al. 2001). They have the capacity to differentiate in vitro into mesodermal and nonmesodermal lineages. One of the cell type obtained in vitro was neuron-like cells (Safford et al. 2002; Ashjian et al. 2003; Dhar et al. 2007; Anghileri et al. 2008; Cardozo et al. 2010; Jang et al. 2010); and in vivo may contribute to functional benefits in a wide range of neurological insults (Kang et al. 2003; Kim et al. 2007; Kulikov et al. 2008; Wei et al. 2009; Chi et al. 2010). This transdifferentiation to nonmesodermal lineages of hASCs is supported by the expression of genes across the three germ layers (Boquest et al. 2005; Katz et al. 2005; Peroni et al. 2008).

The most common techniques used for differentiating ASCs into the neural lineage involve treatment of the cells with retinoic acid (RA), butylated hidroxyanisole (BHA), different growth factors, inhibition of BMP signaling and growth at low density (Safford et al. 2002; Zuk et al. 2002; Ashjian et al. 2003; McCaffery et al. 2003; Dhar et al. 2007; Anghileri et al. 2008; Cardozo et al. 2010; Jang et al. 2010).

Unlike the normal differentiation of NSCs into neurons, however, the differentiation of hASCs into neuron-like cells is essentially a cross-lineage transdifferentiation process. The mechanisms underlying neuronal lineage commitment induced by these techniques remain unclear.

Differentiation is normally associated with an upregulation of transcripts required for the specialized functions of the differentiated cell and a corresponding downregulation of transcripts required for proliferation or for alternative lineages. It is important to have a thorough understanding of the specific signals dictating cellular behavior and the specific cues that induce or inhibit differentiation, and/or promote the maintenance of these stem cells.

Decisions regarding self-renewal vs. commitment are based on microenvironmental cues, which predominantly use the Notch, Wnt, BMP, and Shh signaling pathways (Reynolds and Weiss 1992; Lyden et al. 1999; Gaiano and Fishell 2002; Cai et al. 2008). Stem cell signaling network, especially Wnt, Notch, and BMP signaling cascades are implicated in the regulation of the balance for neural stem cell, progenitor cells, and differentiated neural cells (Israsena et al. 2004; Akai et al. 2005).

One candidate pathway for neural lineage commitment by ASCs is the Wnt/β-catenin pathway that has been shown to be an inducer of neurogenesis (Dorsky et al. 1998; Baker et al. 1999; Patapoutian and Reichardt 2000). Wnt proteins are secreted glycoproteins that regulate development, cell proliferation, cell-fate determination, axonal growth, remodeling, and synaptogenesis in developing neurons (Cadigan and Nusse 1997; Dale, 1998; Hall et al. 2000; Huelsken and Behrens 2002; Salinas, 2003). Whether they promote self-renewal of stem cells (Willert et al. 2003) or fate decisions seems to depend on the specific Wnt molecule and cell type (Lee et al. 2004).

Wnts bind to receptors of the Frizzled family on the plasma membrane to initiate the canonical pathway (Ling et al. 2009). Canonical Wnt signal induces the assembly of Frizzled–Disheveled (Fzd-Dvl) and LRP5/6-Axin complexes to releases β-catenin from the glycogen synthase kinase-3β (GSK-3β) degradation complex. Phosphorylation of the β-catenin by GSK-3β is inhibited by Dvl, causing β-catenin stabilization and accumulation, before translocation to the nucleus, where it binds with members of the T cell factor, lymphoid enhancer factor, and Sox family (TCF/LEF/SOX), to induce expression of target genes (Morin et al. 1997; Jiang and Struhl 1998). Myc, CyclinD1, Axin2, Fgf20, Wisp1, Jag1, and Dkk1 are target genes of this canonical cascade (He et al. 1998; Ishitani et al. 1999; Dejmek et al. 2006; Katoh and Katoh 2006).

Wnt signaling plays a pivotal role in the regulation of proliferation and differentiation of neural progenitors cells, hematopoietic and mesenchymal stem cells (HSCs and MSCs) (Zechner et al. 2003; Etheridge et al. 2004). Several studies have suggested that canonical Wnt signaling keeps stem cells in a self-renewing and undifferentiated state (Reya et al. 2003; Cho et al. 2006). So, Wnt signaling appears to regulate proliferation and differentiation of neuronal lineages in a stage-specific and cellular context-dependent manner (Kasai et al. 2005).

The Notch pathway is a signaling mechanism that controls cell-fate decisions, proliferation and apoptosis during development and in the adult (Artavanis-Tsakonas et al. 1999; Lai, 2004). In mammals, Notch proteins comprise a family of transmembrane receptors. Specific transmembrane ligands interact with Notch receptors on neighboring cells, inducing the cleavage of the Notch intracellular domain (NCID) by gamma secretase, resulting in the release and nuclear translocation of NCID (Artavanis-Tsakonas et al. 1999; Bray, 2006). Nuclear NCID interacts with the transcriptional repressor RBP-Jk (RBP-J/CSL/CBF1/Su(H)/Lag1), and converts it into an activator, leading to the expression of direct Notch target genes (Kageyama et al. 2005; Hurlbut et al. 2007). The best characterized transcriptional targets belong to the hairy/enhancer of split (HES) and the HES-related repressor protein (HERP), which are basic-loop-helix transcription factors that negatively regulate the expression of downstream target genes, such as tissue specific transcription factors, regulating lineage-specific differentiation (Iso et al. 2003; Fischer and Gessler 2007).

The outcome of Notch signaling is highly dependent on the cellular context. Notch activity affects differentiation, proliferation, and apoptotic programs in concert with other cell-intrinsic or cell-extrinsic developmental cues that are necessary to execute specific developmental programs (Artavanis-Tsakonas et al. 1999). The delicate balance between stem cell self-renewal and differentiation has to be tightly controlled by a network of signaling routes, in which Notch cross talks with several other pathways, such as Wnt (Duncan et al. 2005).

Several stem cell types were shown to express elements of the Notch pathway (Karanu et al. 2003; Walsh and Andrews 2003; Duncan et al. 2005; Androutsellis-Theotokis et al. 2006; Chiba, 2006). Its function is necessary to maintain an undifferentiated state and to prevent lineage commitment. In fact, inhibition of Notch signaling leads to a higher rate of differentiation. Then, Notch signaling is required for the prevention of lineage commitment and differentiation (Duncan et al. 2005). The apparent tendency of Notch to inhibit differentiation has suggested that this pathway is an indirect regulator of cell fate, rather than a direct or "instructive" regulator.

During vertebrate neural development, Notch signaling is used to maintain a pool of uncommitment precursors, while a subset of cells is selected to leave this pool and differentiate into neurons. This balance between progenitor

maintenance and neuronal differentiation allows the continuous generation of neurons throughout development and permits temporal control over the specification of distinct neuronal fates (Lewis 1996; Gaiano and Fishell 2002).

Notch and Wnt pathways are important regulators of progenitors and stem cells function. But whether these signals influence distinct elements of self-renewal, such as proliferation or inhibition of differentiation, and how these signals are integrated with one another remains less clear.

In our previous work, we studied Shh and BMP signaling before and after neural differentiation of human adipose tissue stem cells, discovering important findings and similarities between the neural differentiation of these cells and neurogenesis (Cardozo et al. 2010). For this reason, in the present work we analyzed the differential gene expression of Wnt and Notch signaling trying to find a parallelism between neural differentiation of hASCs and neurogenesis.

Materials and Methods

Cell Isolation and Culture

After informed consent and approval of ethics committee of research protocols from Hospital Italiano de Buenos Aires, adipose tissue samples were obtained during abdominal and mammary plastic surgeries of 23 healthy donors between 26 and 56 years old. The adipose tissue was extensively washed with Hank's balanced salt solution (Sigma, Argentina) to remove blood, fibrous material and vessels were carefully dissected and discarded. The remaining tissue was finely minced and digested with 0.1% of Collagenase Type I (Gibco, USA) at 37°C for 45 min with gentle agitation. Enzyme activity was neutralized with a twofold volume of standard medium containing Dulbecco's modified Eagle's medium (DMEM; Gibco) with 20% of fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin (Gibco), and centrifuged for 12 min at 400×*g*. The supernatant containing the lipid droplets was discarded. The stromal vascular fraction (SVF) settled at the bottom was resuspended in standard medium and seeded in culture dishes (Nunc International, Denmark). SVF cultures were incubated at 37°C in a 5% CO_2 atmosphere. After 48 h, nonadherent cells were removed. When they reached 70–80% of confluence, adherent cells were trypsinized (0.25% at 37°C for 5 min, Sigma), harvested, and washed with standard medium to remove trypsin and were then expanded in larger dishes. A homogenous cell population of hASCs was obtained after 2 or 3 weeks of culture. Cells at early passages (3–5) in culture were used for the experiments.

Neuronal Differentiation

Neuronal differentiation of 12 samples was initiated at passages 3–5 using a modification of previous neuronal induction protocols (Woodbury et al. 2000; Zuk et al. 2002; Levy et al. 2003; Tao et al. 2005; Mareschi et al. 2006). Briefly, the cells were plated in dishes until they were subconfluent. Preinduction was performed for 48 h after discarding the medium, washing the cells, and adding new DMEM containing 20% FBS and 1 mM β-mercaptoethanol (Riedel, Germany). Then, the preinduction medium was removed and the induction medium was added to the culture. The composition of induction medium was: DMEM with 100 μM butylated hydroxyanisole (BHA, Sigma), 10–6 M retinoic acid (RA, Sigma), 10 ng/ml epidermal growth factor (EGF, Invitrogen, Brazil), and 10 ng/ml basic fibroblast growth factor (bFGF, Invitrogen). Cells were incubated in this medium during 14 days. The medium was changed every 3 days. The cells were monitored continually after neuronal induction and were lysed for RNA extraction or fixed for immunostaining. One noninduced culture dish was also analyzed with every experiment as a control.

RNA Isolation and Quantitative Real-time PCR

Quantification was performed using real-time polymerase chain reaction (PCR) to compare the levels of expression of Wnt and Notch signaling genes involved in proliferation and neuronal determination. For this purpose, between six and twelve samples of induced and noninduced hASCs were analyzed. Total RNA from hASCs before and 14 days after neuronal induction was extracted using TRIzol Reagent (Invitrogen) according to the manufacturer's recommendations. The purity and integrity of the extracted RNA were evaluated by optical density measurements (260:280 nm ratios) and by visual observation of samples electrophoresed on agarose (Biodynamics, Argentina) gels. Two micrograms of each total RNA were treated with RQ1 RNase-free DNase (Promega, USA) to eliminate possible contamination of genomic DNA. One microgram of treated RNA was used as template in a 20-μl volume cDNA synthesis reaction. Primer sequences were designed using LightCycler Probe Design Software 2.0 (Roche Applied Science, Mannheim, Germany) using gene sequences obtained from the GenBank database (Table 1).

Quantitative real-time PCR was performed using SYBR Green (Invitrogen), Platinum Taq Polymerase (Invitrogen), and LightCycler 2.0 Instrument (Roche Applied Science). The expression of human β-actin was used to standardize gene expression levels. Each sample was run four times. Control experiments without template cDNA revealed no nonspecific amplification. When PCR results were nega-

Table 1 Primers used for real-time PCR experiments

Gene	GenBank	Forward	Reverse	Annealing T° (°C)
Wnt1	NM 005430.3	ACGAGTTTGGATGTTGTA	AGAAAGGAGAGAAGAGTG	57
Wnt5a	NM 003392.3	ATCCCATTCACAGGTTCTC	CTCAAATAGGTTGTCTGCTCT	58
Dkk1	NM 012242.2	ACCAAAGGACAAGAAGGTT	TGGACCAGAAGTGTCTAGCA	57
Wisp1	NM 080838.1	CTGTGAGTGCTGTAAGATGT	TCCTATTGCGTACCTCGG	56
CyclinD1	NM 053056.2	GGTGTCCTACTTCAAATG	CTCCTCGCACTTCTGTTC	58
Notch1	NM 017617.3	TCTITGTTTCAGGTTCAGT	CCTACATTTCAAGAACGG	58
CBF1	NM 005349.2	ACCAGTGTCACATCATCTA	ACACTCAAGTCAGTTTAAG	58
Dtx1	NM 004416.2	ACTCCAATGGCAACAAGGA	CGGATGGTCTGGGTATCAG	57
Hes1	NM 005524.2	AGAGGCGGCTAAGGTGTTT	CTGGTGTAGACGGGGATGA	58
Herp1	AF232238.1	GCAATAGTAACTGAATGTCCT	GGCATCTGTATGGCTACC	58
Hey1	NM 012258.3	CTATCGGAGTTTGGGATTTCG	GGGTCAGAGGCATCTAGTC	58
β-Actin	NM 001101	CCCTTGCCATCCTAAAAGC	TGCTATCACCTCCCCTGTGT	57

tive, cDNAs from human cell lines or tissues were run as positive controls in order to eliminate the possibility of false negative results. To verify the identity of amplified DNAs, the size of the PCR products was checked on agarose gel.

Statistical Analysis

All data are presented as mean±error deviation. The values obtained from the real-time PCR were analyzed with Relative Standard Curve method and the error deviations were obtained according to the Applied Biosystems User Bulletin No. 2 (P/N 4303859).

Statistical comparison of the results obtained with induced and noninduced hASCs was carried out according to the Student's *t* test (to compare two treatment groups). Differences were considered statistically significant when $p<0.05$. Statistical analysis was performed using the program Primer of Byostatistics version 5.0 (McGraw-Hill, 2002).

Results

hASCs Cell Characterization

As we have previously described (Cardozo et al. 2010), the SVF exhibited a heterogeneous cell population with different morphologies that included spindle-shaped, large, flat and small round cells. With continued cell growth and division in culture, the spindle-shaped cells gradually become predominant. Immunocytochemistry analysis of hASCs demonstrated that the cells were negative for CD34 and CD45, cell surface markers associated with hematopoietic cells. In contrast, hASCs expressed CD90 and Stro-1, two typical markers of mesenchymal stem cells (data not shown). Thus, we obtained a mesenchymal stem cell population with morphology, plasticity and expression of the characteristic membrane markers.

Changes after Neuronal Induction

To induce neuronal phenotype, hASCs were maintained in subconfluent cultures. After the neuronal induction, the morphology of hASCs began to change, cells changed from flat, elongated, spindle-shaped to round cell bodies with several branching extensions and retractile characteristics similar to those observed in cultured neuronal cells. As we have previously shown (Cardozo et al. 2010), we detected the expression of the neural markers βIII-tubulin, Tau, NF-200, synaptophysin, and GFAP after the differentiation and induced cells stained with the recycling synaptic vesicles dye FM1-43 (Table 2). No labeling was detected in noninduced cells.

Taken together, these data indicate that exposure to neuritizing cocktails induces process outgrowth, expression of neuroglial markers, and membrane excitability in hASCs;

Table 2 Summary of immunocytochemical profile in neuronal-induced and noninduced hASCs demonstrated by immunocytochemistry and Western blot

Marker	Induced hASCS	Noninduced hASCs
Nestin	+	+
GFAP	+	+
S100	−	−
βIII-tubulin	+	−
NeuN	−	−
NF200	+	−
Synaptophysin	+	−
Tau	+	−
FM1-43	+	−

but electrophysiological studies are necessary to support that these cells are competent to differentiate into mature and functional neurons upon exposure to neuritizing stimuli.

Wnt Signaling in Neuron-Like Phenotype Cells

To gain insight into the molecular mechanisms involved in proliferation and neuronal differentiation of hASCs, we evaluated the expression before and after 14 days of neuronal induction of Wnt signaling genes known to have important roles during proliferation and differentiation (Baek et al. 2003; Reya et al. 2003; Zechner et al. 2003; Etheridge et al. 2004; Kasai et al. 2005; Cho et al. 2006). Using quantitative real-time PCR, induced and noninduced hASCs gene expression profiles of the following factors were compared: Wnt ligands (Wnt1 and Wnt5a), inhibitor (Dkk1), and target genes (CyclinD1, Wisp1).

We detected the presence of the canonical Wnt signaling in hASCs, which probably is involved in proliferation of these cells. Wnt5a, CyclinD1, Wisp1, and Dkk1 expression were detected in hASCs but expression of Wnt1 was not observed in these cells using this technique. After neuronal induction, it was a downregulation of the cascade's transcripts (Fig. 1), according with previous works were Wnt signaling was associated with the control of "stemness" of mesenchymal stem cells (Etheridge et al. 2004). But we could not detect an upregulation of its inhibitor (Dkk1) which probably is involved in the control of the determination of the different lineages.

Notch Signaling in Neuron-Like Phenotype Cells

Given the well-documented role of Notch signaling in regulating proliferative capacity, cell-fate acquisition, and differentiation of neural stem cell populations (Lewis, 1996; Gaiano and Fishell 2002; Duncan et al. 2005; Chiba 2006), we investigated the presence of Notch pathway transcripts (Notch1, CBF1, Dtx1, Herp1, Hes1, and Hey1) by real-time PCR in hASCs before and 14 days after neuronal induction.

We found expression of transcripts for elements functioning at distinct levels of the signaling cascade in uninduced hASCs. Notch1, CBF1, Hes1, Herp1, and Hey1 transcripts were expressed in hASCs. Dtx1 were expressed at undetectable levels.

After neuronal induction, it was detected a statistically significant decrease ($p<0.05$) of the expression of all Notch signaling transcripts analyzed (Fig. 2).

Taken together, mRNA expression of Notch pathway components was differentially regulated by neuronal differentiation in hASCs. There was an active Notch pathway in hASCs according to the function of this cascade in maintain the self-renew of these cells and a downregulation of it after the neuronal induction, necessary to the commitment of these cells to the neural lineage.

Discussion

To date, differentiation into neuron-like cells of hASCs has been promoted by adding various factors, cytokines or

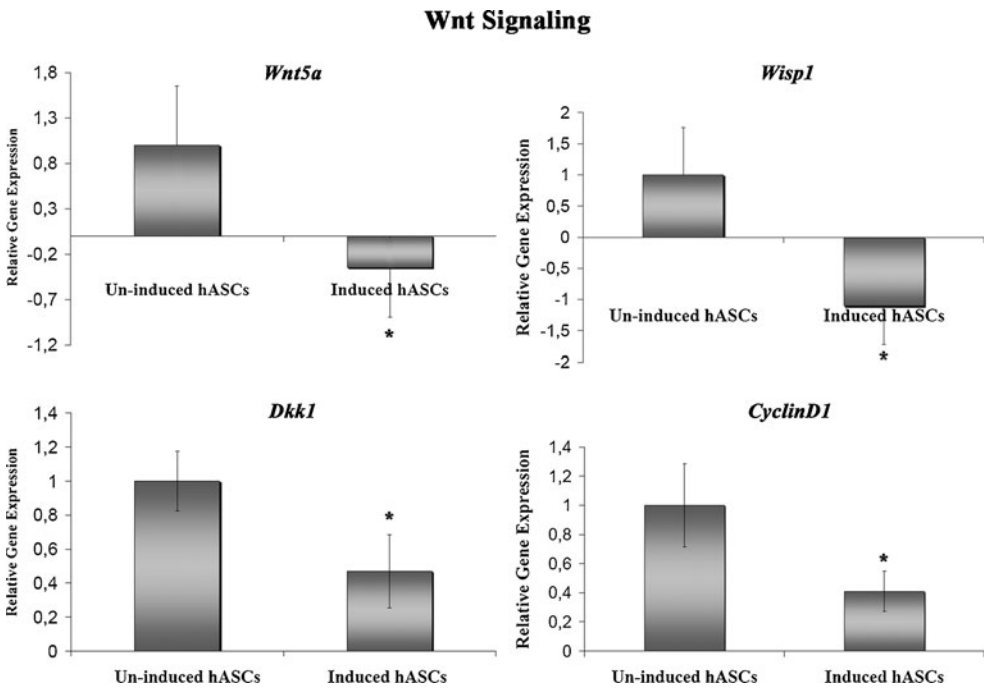

Fig. 1 Wnt signaling is downregulated in hASCs after neuronal differentiation. hASCs were cultured in neuronal induction medium during 14 days. Real-time PCR analysis shows decreased mRNA levels of Wnt5a, Wisp1, Dkk1, and CyclinD1 in induced hASCs relative to noninduced hASCs. Relative gene expression of each gene (mean±error deviation), normalized to the relative expression of the housekeeping gene β-actin, for induced and noninduced hASCs is shown. *$p<0.05$, significantly different from control

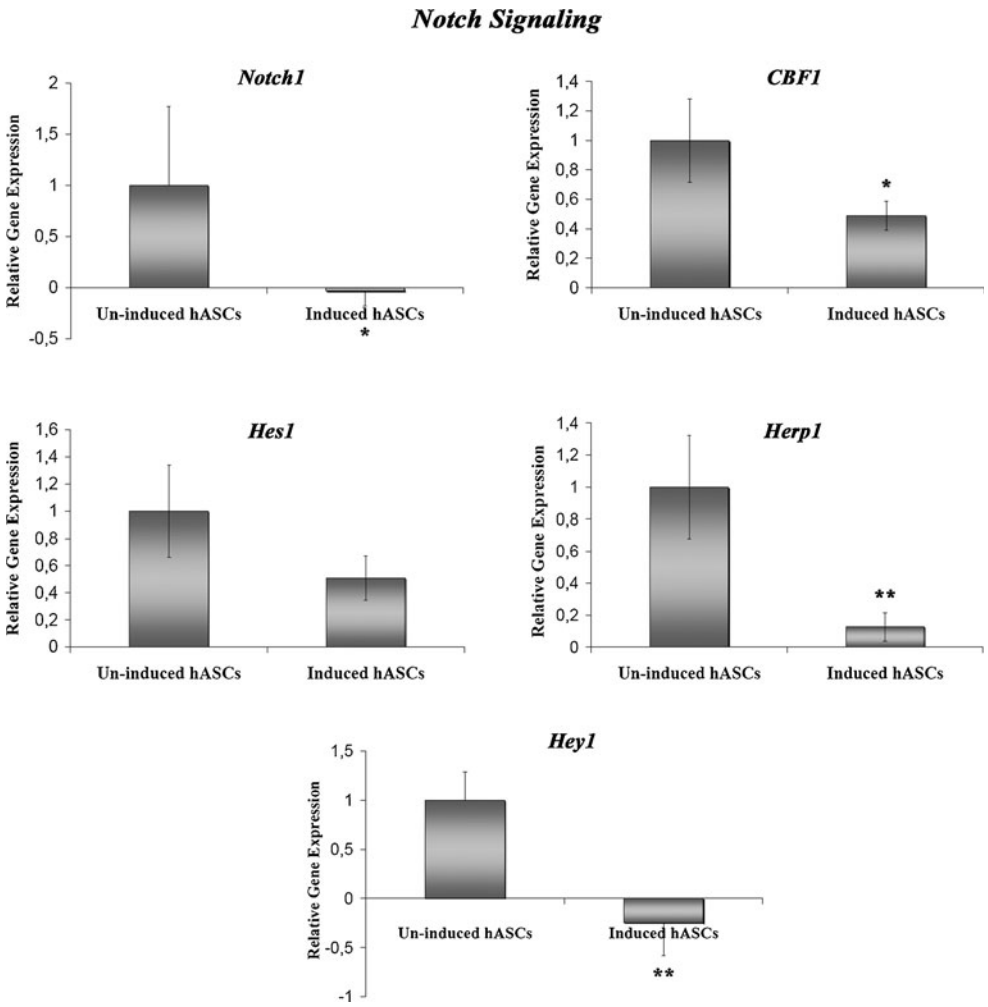

Fig. 2 Notch signaling is downregulated in hASCs after neuronal differentiation. Real-time PCR analysis shows a decrease of Notch1, CBF1, Hes1, Herp1, and Hey1 mRNA levels in hASCs that were cultured in neuronal induction medium during 14 days relative to noninduced hASCs. Relative gene expression of each gene (mean±error deviation), normalized to the relative expression of housekeeping gene β-actin, for induced and noninduced hASCs is shown. *$p<0.05$; **$p<0.01$, significantly different from control

antioxidants (Safford et al. 2002; Ashjian et al. 2003; Dhar et al. 2007; Anghileri et al. 2008; Cardozo et al. 2010; Jang et al. 2010). The efficiency of these strategies, however, was not sufficient enough for therapeutic applications. If we could elucidate the underlying molecular mechanism of neuronal differentiation of hASCs, it may become possible to increase the efficiency sufficiently. Furthermore, hASCs may provide an easily obtainable, reliable source of autologous stem cells; it could solve the problems such as histocompatibility, inadequate tissue supply and ethical concerns existing when NSCs and ESCs were used in cell therapy. In the present study, we have detected the presence of Wnt and Notch signaling pathways in hASCs proliferating cells and the decrease of both cascades after neuronal differentiation.

This work has shown expression of several components of Wnt signaling pathway by hASCs, which suggests that this signaling pathway may be of fundamental importance in these cells. It is clear that more specific techniques to manipulate particular components of the canonical Wnt signaling pathway are necessary to determine more precisely how Wnt signaling participates in neuronal differentiation of hASCs. However, our findings support previous data, which suggest that Wnt signaling may regulate differentiation pathways in various mesenchymal cell types (Ross et al. 2000; Bennett et al. 2002; Etheridge et al. 2004) Endogenous canonical Wnt signaling in preadipocytes and BMSCs inhibits further differentiation and spontaneous differentiation occurs following inhibition of this pathway. Wnt expression levels are high in preadipocytes or BMSCs, but decrease when differentiation is stimulated.

However, activated Wnt signaling has also been shown to promote neurogenesis; for example, Wnt5a was a weak mitogen; it efficiently promoted the acquisition of a DA phenotype in neural precursors (Castelo-Branco et al. 2003). The functional diversity of the various Wnt proteins is thought to be due, at least in part, to the specific Fzd with which they bind and the particular signaling cascade that is subsequently stimulated within the cell. It now appears likely that individual Wnts are able to bind multiple Fzd receptors, and each receptor can bind numerous Wnt proteins (Dale 1998). Stimulation of a pathway by a particular Wnt also appears to depend on the experimental system employed, a specific ligand could signal via both

canonical and noncanonical mechanisms (Hartmann and Tabin 2000). Therefore, the specific intracellular pathways stimulated by each of the Wnt proteins expressed by hASCs need to be established to fully understand the signaling mechanisms involved in regulating neuronal differentiation.

In the present work, we used bFGF and retinoic acid as neuritizing agents and it is important to note the interactions of these agents with the Wnt signaling. FGF2 treatment of neural progenitor cells provides mitogenic and trophic support and also influences progenitor cell-fate choice (Maric et al. 2003). Members of the Wnt family are usually found in the proximity of high FGF synthesis domains during neural development and act in cooperation with FGFs in controlling cell fate (Viti et al. 2003). FGF2 increases the pool of β-catenin, which plays an important role in maintaining neural potential by directly and indirectly controlling expression of proneural genes (Israsena et al. 2004). It has been reported that retinoic acid treatment can induce P19 cells to become neurons, and Wnt signaling it is involved in this process with an increase of the level of both nuclear and cytosolic β-catenin (Papkoff 1994; Tang et al. 2002). While β-catenin has mitogenic effects, it is not by itself sufficient to maintain cells in the proliferative state and requires other growth factors like serum. In situations where serum is limited, the proneuronal effects of β-catenin predominate in pluripotent and multipotent cells. β-catenin can activate a number of fate determination genes of the bHLH family and that this effect is very likely to play an important role in the regulation of stem cell lineage commitment (Israsena et al. 2004). β-catenin binds directly to the promoters of several proneural genes and activates their expression, which may underlie the proneural effects of it.

Notch has been widely studied in the context of stem cells, hoping that specific modulation of the signal may provide an in vitro tool for stem cells expansion and for the manipulation of lineage-specific differentiation in the future. We demonstrated that key Notch signaling components are present in hASCs. Notch1, CBF1 and the target genes Hes1, Herp1, and Hey1 were detected, allowing us to conclude that hASCs posses the necessary elements required for a functional Notch signal and that this signal is downregulated after 14 days of neuronal differentiation. Hes1, Herp1, and Hey1 act as repressors of bHLH transcription factors to regulate lineage commitment by inhibiting expression of cell-fate-specific genes, thereby maintaining stem/progenitor cells. These results are similar to those obtained in a previous work in HSCs (Duncan et al. 2005; Yu et al. 2006), where it was demonstrated that Notch signaling is used by HSCs and is downregulated as these cells differentiate; and in NSCs, targets of Notch signaling work together to prevent terminal differentiation and preserve a pool of stem cells, downregulating proneural genes such as Mash1 (Alexson et al. 2006). Based on these data, we could suggest that Notch1 and they target genes, key gatekeepers of progenitor populations and regulators of glial and neural stem cell fates (Artavanis-Tsakonas et al. 1999; Gaiano and Fishell 2002), probably regulate the proliferation and differentiation-induced expression of neural-glial markers in hASCs, supporting the notion that hASCs differentiation into neuron-like cells represents a regulated process analogous to what occurs during normal neuronal differentiation of NSCs.

If these two signaling pathways are interconnected in hASCs is unclear. In HSCs, Notch signaling is required for the influence of Wnt, probably Wnt signaling exerts its influence by activating the Notch pathway, but it is also possible that represent parallel pathways, with Wnt enhancing proliferation and Notch preventing differentiation (Duncan et al. 2005).

Future studies elucidating the function of Wnt and Notch signaling in fate specification and the relationship between them in hASCs will lead to a more thorough understanding of the stem cell biology, which hopefully will result in the development of practical applications in cell therapy.

In summary, while it is unlikely that hASCs will be able to directly replace lost neurons and restore function to neuronal circuits through mechanisms that involve hASCs adopting and maintaining robust neuronal phenotypes, hASCs may still be able to contribute to neural repair through other mechanisms. Additional studies are needed to determine the extent to which autologous transplants of hADSCs at an injury site survive, engraft, and interact with endogenous progenitor populations to enhance neural repair.

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