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Transcription factors expressed in embryonic and adult olfactory bulb neural stem cells reveal distinct proliferation, differentiation and epigenetic control☆

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ABSTRACT

TF genomic markers associated with neurogenesis, proliferation, differentiation, and epigenetic control in human embryonic neural stem cells (hENSC(, and adult human olfactory bulb neural stem cells (OBNSC) were studied by immunohistochemistry (IHC) and DNA microarray. The biological impact of TF gene changes in the examined cell types was estimated using DAVID to specify a different GO class and signaling pathway based on KEGG database. Eleven, and twenty eight TF genes were up-regulated (fold change ≤2–39) in OBNSC, and hENSC respectively. KEGG pathway analysis for the up-regulated TF genes revealed significant enrichments for the basal transcription factor pathway, and Notch signaling pathway in OBNSCs, and hENSCs, respectively. Immunofluorescence analysis revealed a significantly greater number of β-tubulin III (TUBB3), MAP, glial fibrillary acidic protein (GFAP), and O4 in hENSC when compared to those in OBNSC. Furthermore, the expression of epigenetic-related TF-genes SMARCC1, TAF12, and UHRF1 increased significantly in OBNSC when compared with hENSC.

1. Introduction

Neural stem cells (NSC) are multipotent, self-renewal cells isolated from adult human olfactory bulb (OB) [1], and fetal brain tissue [2]. They could also be obtained by differentiation of human embryonic stem cells (hENSC) using sets of well-defined protocols [3]. Isolation of NSCs from adult human OB represents an accessible source of neural precursors [4], and is expected to provide an attractive tool for transplantation-based therapy of neurodegenerative diseases that avoids the ethical issues raised by the use of human embryos [5].

An increasing amount of information about the possible roles of transcription factors (TFs) in regulation of various biological processes of NSCs [6], is making it possible to utilize TF gene expression profile to explore variations in proliferation and differentiation potentiality of NSCs.

Several TFs had been implicated in NSC lineage specification. In this respect, Myt1 is found to be expressed in proliferating neural progenitors, oligodendrocyte progenitor cells, and differentiating neurons [7]. Pax6 and Ascl1 regulate cohorts of genes that promote self-renewal, basal progenitor cell genesis and neurogenesis [8]. Ascl1, POU3F2, and Myt1L directly reprogram fibroblasts into induced dopaminergic tyrosine hydroxylase (TH) positive neurons [9]. At the translational level, direct reprogramming of somatic cells and NSCs with predefined sets of TF genes provides a fundamentally new approach for the generation of patient-specific cells for *in vitro* disease modeling or direct therapeutic applications [10], and highlights the significance of TF gene regulation in determining cell fate.

In our previous studies, we highlighted the global gene expression profile of adult human OBNSC, human embryonic NSC, and substantia nigra cells which are known to be rich in dopaminergic neurons [11,12]. In most cases, variation in global gene expression between different cell lineages is reflected in the expression profile of their TFs. This strategy might lead us to restrict the global gene expression profile of these cells from whole human genome (DNA chip content) to a fewer number of decisive TF genes.

Although there is a great interest and potential of adult and embryonic NSC's in cell replacement therapy, there is lack of data about their TF gene expression profiling, and molecular pathways that govern their proliferation, and differentiation potential. Moreover, revealing possible exposure of adult and embryonic NSC to epigenetic changes is crucial to explore their future therapeutic potentials. A better understanding of the molecular basis of the aforementioned processes would facilitate choice of the best cell source for different neurodegenerative and traumatic CNS insults.

To this end, this study was designed to highlight possible variations in proliferation and differentiation potentials of adult human OBNSC and hENSC as well as their possible exposure to epigenetic marks by examining their TF gene expression and immunohistochemical profiles.

2. Materials and methods

2.1. Ethical issue

For the human embryonic NSC, five different (not replicate) cell samples were purchased from Invitrogen derived from NIH-approved

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H9 (WA09) human embryonic stem cells (hESCs). For the adult OBNSC, written consents for carrying out the operations were obtained from the patients undergoing brain surgery. Verbal consents for the probability of isolating NSC from the collected OB tissue were also obtained from the patients. Written and verbal consent were approved by the Ethical Committee, Catholic University, School of Medicine, Rome, Italy, and Ethical Committee of Mansoura University, Egypt.

2.2. Culturing of human embryonic NSCs

Cryopreserved human embryonic neural stem cells were plated in a 6-well culture plate coated with polyethyleneimine, and incubated at 37 °C in a 5% CO_2/95% air incubator in serum-free DMEM/F12 medium (Invitrogen, Carlsbad, CA, USA) supplemented with a mixture of insulin–transferrin–selenium (ITS) (Invitrogen, Carlsbad, CA, USA), 20 ng/ml recombinant human EGF (Invitrogen, Carlsband, CA), 20 ng/ml recombinant human bFGF (Invitrogen, Carlsband, CA), and 10 ng/ml recombinant human LIF (Invitrogen, Carlsband, CA), according to the methods described previously [13]. The half of the medium was renewed every 4 days. Following incubation for several months, the embryonic NSC in culture continued to proliferate by forming free floating or loosely attached growing spheres. For microarray analysis, nonpassage embryonic NSC spheres were harvested, replated in a noncoated 6-well culture plate, and incubated further for 72 h in the NSC medium without inclusion of 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA).

2.3. Isolation and culturing of human olfactory bulb NSCs

Immediately after removal, the OBs were dissociated in Papain 0.1% (Sigma-Aldrich, St. Louis, MO) for 30 min at 37 °C. Dissociated cells were cultured in the presence of human recombinant EGF (20 ng/ml; PeproTech, Rocky Hill, NJ), human recombinant bFGF (10 ng/ml; PeproTech), and LIF (20 ng/ml; Immunological Sciences, Rome, Italy) in DMEM/F12 (1:1) serum-free medium (Invitrogen, Carlsband, CA) containing L glutamine 2 mM, glucose 0.6%, putrescine 9.6 μg/ml, progesterone 0.025 mg/ml, sodium selenite 5.2 ng/ml, insulin 0.025 mg/ml, apo-transferrin sodium salt 0.1 mg/ml, sodium bicarbonate 3 mM, Hepes 5 mM, BSA 4 mg/ml, and heparin 4 μg/ml. Primary neurospheres were dissociated with Accutase (Invitrogen) for 4 min at 37 °C, serially diluted and plated one cell per mini-well onto 96-well plates. Mini-wells containing one single cell were marked after microscopic confirmation and assessed for secondary neurosphere generation after one week. Secondary neurospheres were subsequently dissociated, plated at the density of 10^3 cells/cm^2 in serum-free medium containing EGF and bFGF, and passaged up to P30. Between P7 and P10, parallel cultures were established in which cells were grown as adherent monolayers in medium containing EGF and bFGF supplemented with 5% fetal calf serum (Hyclone, Logan, UT). Cells were counted with hemacytometer every 48 h. Differentiation assays (the same for hENSC) were performed by 14 days after plating on Matrigel coated glass coverslips in the absence of EGF and bFGF and in the presence of 1% fetal calf serum (Hyclone) supplemented with 3′–5′-cyclic adenosine monophosphate (cAMP) 50 mM, all-trans retinoic acid 5 mM (Sigma Aldrich), and triiodothyronine (T3) 30 nM (Sigma Aldrich) [14].

2.4. Illumina bead chip hybridizations and analysis of expression data

Total cellular RNA was isolated from proliferating human embryonic NSC (n = 5, passage 2, different samples not replicates), and adult human OBNSCs (n = 5, passage 6, different samples not replicates) using the Trizol (Invitrogen). Biotin labeled cRNAs were generated from total RNA using a linear amplification kit (Ambion, Austin, TX, United States), and their concentrations were confirmed with Nanodrop ND-1000. The quality control step was performed using a BioRad Experion electrophoresis station. Illumina SentrixH HumanHT-12 v3 Expression Bead Chips were used. About 800 ng cRNA samples were hybridized onto the specified chips at 58 °C overnight (19 h), scanned, and the numerical results were extracted with GenomeStudio using the Gene Expression Module v.1.0.6. Raw data were normalized using the quantile normalization method (lumi software package) [15]. Normalized data were filtered for genes with significant expression levels compared to negative control beads. Selection for differentially expressed genes was performed on the basis of arbitrary thresholds for fold changes plus statistical significance according to the Illumina *t*-test error model (limma software) [16]. The mRNA array data is MIAME compliant and has been submitted to the NCBI Gene Expression Omnibus (GEO) database (accession: under processing).

2.5. Data analysis

2.5.1. TF genes differential expression analysis

We identified Gene Ontology (GO) groups of genes whose expression was differentially regulated among the classes. The evaluation of which Gene Ontology classes are differentially expressed between different classes was performed using a functional class scoring analysis as described by Pavlidis et al. [16]. For each gene in a GO class, the p value for comparing classes was computed. The set of p values for a class was summarized by two summary statistics: (i) the LS summary is the average log p values for the genes in that class and (ii) the KS summary is the Kolmogorov–Smirnov statistic computed on the p values for the genes in that class. The statistical significance of the GO class containing n genes represented on the array was evaluated by computing the empirical distribution of these summary statistics in random samples of n genes. Functional class scoring is a more powerful method of identifying differentially expressed gene classes than the more common over-representation analysis or annotation of gene lists based on individually analyzed genes. The functional class scoring analysis for Gene Ontology classes was performed using BRB-ArrayTools. Enrichment was determined in reference to all human Entrez GeneIDs that were annotated in the Biological Process branch (14,394 genes total). P-values were derived from a hypergeometric test followed by the Benjamini and Hochberg false discovery rate [17]. A P-value cutoff of 0.01 was used to identify significantly enriched categories.

2.5.2. Functional annotation and molecular network analysis

Functional annotation of significant genes identified by microarray analysis was searched by the web-accessible program named Database for Annotation, Visualization and Integrated Discovery (DAVID) version 2009, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) (david.abcc.ncifcrf.gov) [18]. DAVID covers more than 40 annotation categories, including Gene Ontology (GO) terms, protein–protein interactions, protein functional domains, disease associations, biological pathways, sequence general features, homologies, gene functional summaries, and tissue expressions. By importing the list of the National Center for Biotechnology Information (NCBI) Entrez Gene IDs, this program creates the functional annotation chart, an annotation-term-focused view that lists annotation terms and their associated genes under study. To avoid excessive count of duplicated genes, the Fisher's exact test is calculated based on corresponding DAVID gene IDs by which all redundancies in original IDs are removed. Gene Ontology (GO) and KEGG molecular pathway analysis was performed to identify possible enrichment of genes with specific biological themes using both the data set as a whole and then in the individual K-means clusters. DAVID calculates a modified Fishers Exact p-value to demonstrate GO or molecular pathway enrichment, where p-values less than 0.05 after Benjamini multiple test correction are considered to be strongly enriched in the annotation category.

2.5.3. Genomic profiling of transcription factor genes

The list of genes with predicted TF-binding sites in their regulatory regions in each of our examined cell class were imported to g:Profiler. The purpose of g:Profiler is to find common high-level knowledge such as pathways, biological processes, molecular functions, subcellular localizations, or shared TF binding sites (TFBS) to the list of input genes. The data used in g:Profiler is derived from the Gene Ontology [19], KEGG [20], Reactome [21] and TRANSFAC [22] databases.

2.5.4. Immunohistochemical validation of selected genes

For immunocytochemistry, proliferating (P2 hENSC, P6 OBNSCs) and differentiating (P3 ENSC, and P9 OBNSC) attached on poly-L-lysine-coated cover glasses were fixed with 4% PFA in 0.1 M phosphate buffer, pH 7.4 at room temperature (RT) for 5 min, followed by incubation with phosphate-buffered saline (PBS) containing 0.5% Triton X-100 at RT for 3 min. After blocking non-specific staining by PBS containing 10% NGS, the cells were incubated in primary antibodies. We used antibodies against nestin (NES) (Chemicon, Temecula, CA), GFAP (Dako, Glostrup, Denmark), β-tubulin III (TUBB3) (Chemicon), neurofilament RT-97 (Developmental Studies Hybridoma Bank, Iowa City, IA), MAP2 (Chemicon), NG2 (Chemicon), and O4 (Chemicon). Then, they were incubated at RT for 30 min with a mixture of FITIC-conjugated anti-rabbit IgG (Invitrogen) and TRITIC-conjugated anti-mouse IgG (Invitrogen). After several washes, they were examined on the Olympus BX51 universal microscope. Immunostaining of NS/PCs was performed.

3. Result and discussion

3.1. Embryonic human NSCs in culture

Passage two Invitrogen hENSC derived from NIH-approved H9 (WA09) human embryonic stem cells (hESCs) were characterized based on the manufacturer's instruction manual. A single cell suspension of hENSC was cultured in serum-free DMEM/F12 medium supplemented with EGF and bFGF. The cells gave rise to proliferating neurospheres, first appearing within 72 h of primary culture and increasing their numbers and diameters quickly during 7 days after the onset of the culture. Approximately 90% of the cells stained positive for the undifferentiated NSC marker nestin (NES), SOX2 and the proliferation marker Ki67 (Figs. 1A,B). The differentiation capacity of hENSC in differentiating conditions was revealed by examining the types of molecular markers expressed by neurons and glial cells in passage 3 hENSC. These cells could differentiate into, Dcx-positive immature neurons (25%) (Fig. 1C), GalC positive oligodendrocyte (5%) (Fig. 1D), and GFAP positive astrocyte (70%) (Fig. 1E).

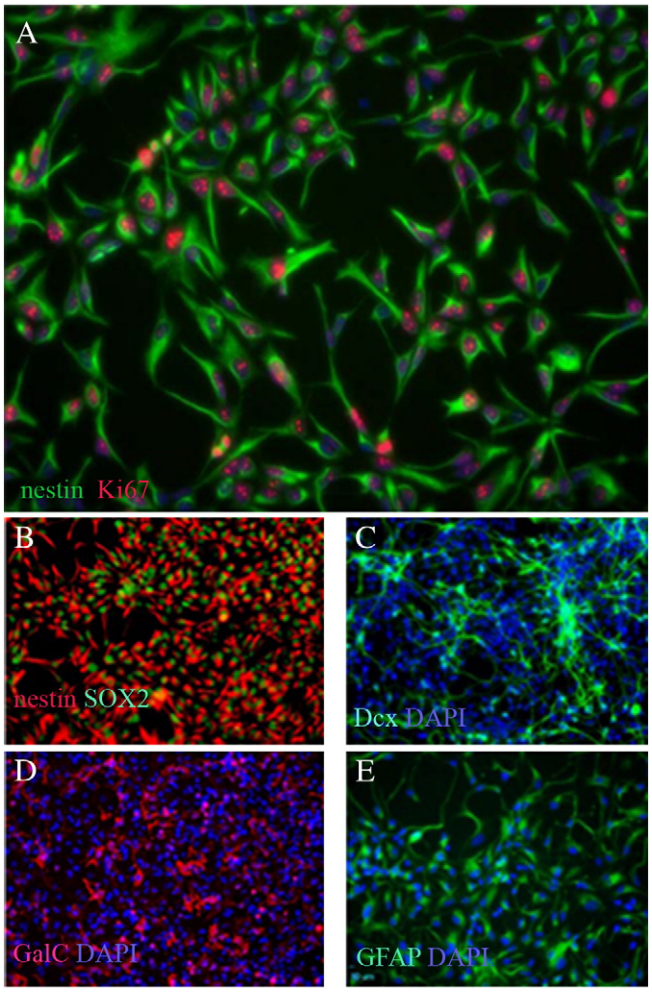

Fig. 1. Fluorescence image (20×) of GIBCOR hNSCs at passage 3 that has been cultured in StemProR NSC SFM and stained for the NSC phenotype marker nestin (NES) (green) and the proliferation marker Ki67 (red, A). Cell nuclei were counterstained with DAPI (blue, A). Approximately 90% of the cells stain positive for the undifferentiated NSC marker nestin (NES) and the proliferation marker Ki67. Lack of Oct4 staining indicates that there are no remnant hESCs in the culture (data not shown) (Invitrogen, manual part no. A11592, MAN0001758). Fluorescence images (20×) of GIBCOR hNSCs that have been cultured in StemProR NSC SFM for three passages, and then allowed to differentiate into neurons, oligodendrocytes, or astrocytes. Upon directed differentiation, cells start to lose the undifferentiated NSC marker, nestin (NES), but stain positive for the differentiated cell type markers Dcx, GalC, and GFAP. Cells were stained for the undifferentiated NSC markers nestin (NES) (red, B) and SOX2 (green, C) prior to directed differentiation. Cells were then differentiated into neurons and glial cells, and respectively stained for the neuronal marker Dcx (green, C), for the oligodendrocyte marker GalC (red, D), or for the astrocyte marker, GFAP (green, E). The nuclei were counterstained with DAPI (blue) in panels B–D (Invitrogen, manual part no. A11592, MAN0001758).

3.2. Adult human OBNSCs in culture

Passage 3 dissociated adult human OB specimens were cultured in serum-free medium supplemented with the mitogens EGF and bFGF. Under these conditions, the OB cells generated primary neurospheres with latencies that ranged from 6 to 8 weeks. When passage 9 adult human OBNSC spheres were split into single cells with Accutase and incubated in the NSC medium supplemented with 10% FBS, they rapidly attached on the plastic surface, and started to differentiate into the different neuronal and glial lineages. The proliferated human OBNSC exhibited an intense immunoreactivity for GFAP astrocyte marker (75%), MAP2 immature neuronal marker (17.5%), and β-tubulin (TUBB3) mature neuronal marker (5%) (Figs. 2A,B). None of the cells expressed the oligodendrocyte marker O4.

From the IHC results, hENSCs differentiated into astrocytes (70%), oligodendrocytes (5%), and neurons (25%). The proliferation and differentiation potentials of adult OBNSC were comparable to those of the embryonic ones where they differentiated into astrocytes (75%), and neurons (17.5%), but not oligodendrocytes, demonstrating that in comparison to hENSCs, OBNSCs had restricted neuronal and oligodendrocyte differentiation *in vitro*, and therefore are expected to be less effective in regard to neuronal and oligodendrocyte replacement therapy. Previous studies demonstrated that adult NSCs are able to differentiate into both glia and neurons, but only under particular growth conditions including co-culturing with embryonic stem cell-derived neural precursors [23]; this suggests that adult NSC appear to lack key factors required for neuronal differentiation. The restricted *in vitro* neuronal differentiation capacity of adult NSCs is supported by other *in vivo* studies where it was demonstrated that following engraftment, adult NSC differentiated into astrocytes and oligodendrocytes, but not neurons [24].

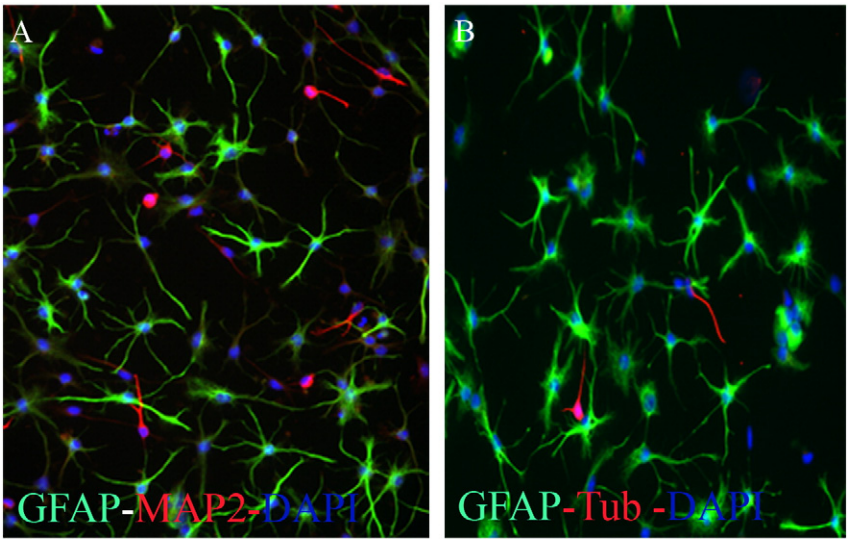

Fig. 2. Differentiation potential of short-term proliferated human OBNS cells. Fluorescent phase contrast images of passage 9 human OBNS immunostained for the GFAP astrocyte marker, MAP2 immature neuronal marker, and β-tubulin (TUBB3) mature neuronal marker. Scale bar, 100 μm. The nuclei were stained blue with DAPI. The plot shows the percentage of positive GFAP astrocytes, MAP2 immature, and β-tubulin (TUBB3) mature neurons, generated by each cell type.

3.3. TF gene expression profile of OBNSC

In order to highlight a possible variation in the proliferation potential of our hENSC, and OBNSCs, we studied the gene expression profile of their TF genes during their proliferation phase, passage 3, and 6 respectively. In passage 6 proliferating OBNSCs, eleven TF genes were up-regulated (fold change ≤ 6–30). The expression of FOXO4, CTNNB1, NFIC, TGIF1, GTF2H4, TSHZ1, HNRNPAB, FOXN2, ZFP36L2, HNRNPK, and TAF12 were up-regulated by 30.1, 25.98, 21.84, 19.98, 17.03, 16.31, 13.88, 11.85, 11.51, 11.41, and 6.38 fold respectively (Fig. 3, Tables S1, S2).

3.4. GO clustering of up-regulated TF genes of OBNSC

GO clustering using DAVID for the 11 up-regulated TF genes (fold change ≤ 6.38–30.1) of OBNSCs revealed three main clusters (Table 1). The first annotation cluster with an enrichment score of 4 include: transcription regulator activity (7 genes), regulation of RNA metabolic process (7 genes), transcription factor activity (6 genes), regulation of transcription (7 genes), DNA binding (7 genes), regulation of transcription (6 genes), DNA-dependent (6 genes), and nucleus (6 genes).

3.5. KEGG pathway analysis of TF up-regulated genes of OBNSC

KEGG pathway analysis of the eleven TF up-regulated genes (fold change ≤ 6.38–30.1) of OBNSCs showed a significant enrichment for basal transcription factor pathway.

3.6. TF gene expression profile of hENSC

In hENSCs, 28 TF genes were up-regulated (fold change ≤ 2.13–39.1) (Tables S1, S2). The expression of ZNF33B, TRIM25, HOXC8, YEATS4, ZHX2, SUPT4H1, LHX2, HOPX, GAS7, RFXANK, ATF6, MSRB2, ETV5, HOXB7, BATF3, KDM5B, HDAC1, HCFC1, TCF25, UHRF1, HNRNPAB, HES6, HDAC2, DRAP1, EDF1, RCAN1, ATF4, and JUN were up-regulated by 2.13, 2.56, 2.68, 3.28, 3.49, 3.81, 4.33, 4.72, 5.41, 5.81, 6.41, 6.76, 6.94, 7.16, 8.64, 8.48, 9.48, 13.95, 14.98, 15, 16.80, 19.29, 21.43, 22.03, 27.28, 29.26, 38.75, and 39.44 fold respectively.

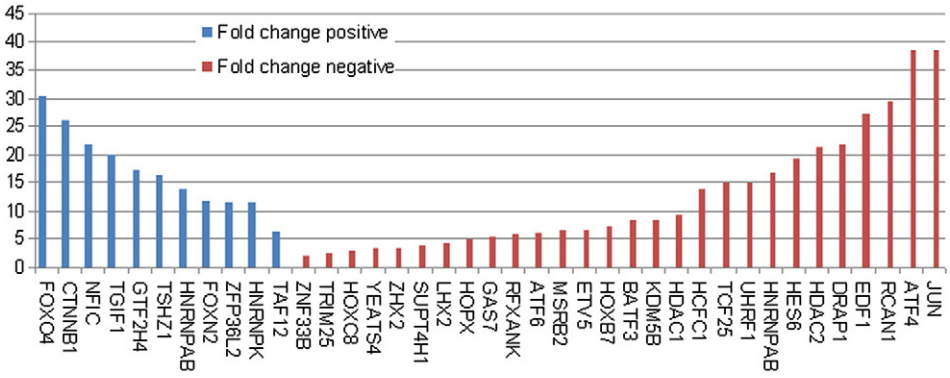

Fig. 3. Up-regulated and down-regulated TF genes between hENSC and OBNSC. Eleven TF genes were up-regulated (fold change ≤ 6–30). The expression of FOXO4, CTNNB1, NFIC, TGIF1, GTF2H4, TSHZ1, HNRNPAB, FOXN2, ZFP36L2, HNRNPK, and TAF12 were up-regulated by 30.1, 25.98, 21.84, 19.98, 17.03, 16.31, 13.88, 11.85, 11.51, 11.41, and 6.38 fold respectively. In hENSCs, 28 TF genes were up-regulated (fold change ≤ 2.13–39.1) (Tables S1, S2). The expression of ZNF33B, TRIM25, HOXC8, YEATS4, ZHX2, SUPT4H1, LHX2, HOPX, GAS7, RFXANK, ATF6, MSRB2, ETV5, HOXB7, BATF3, KDM5B, HDAC1, HCFC1, TCF25, UHRF1, HNRNPAB, HES6, HDAC2, DRAP1, EDF1, RCAN1, ATF4, and JUN were up-regulated by 2.13, 2.56, 2.68, 3.28, 3.49, 3.81, 4.33, 4.72, 5.41, 5.81, 6.41, 6.76, 6.94, 7.16, 8.64, 8.48, 9.48, 13.95, 14.98, 15, 16.80, 19.29, 21.43, 22.03, 27.28, 29.26, 38.75, 39.44 fold respectively.

Table 1
GO clustering using DAVID for the 11 up-regulated TF genes (fold change ≤ 6.38–30.1) of OBNSCs.

Annotation cluster 1	Enrichment score: 4	Count	P value	Benjamini
GOTERM_MF_FAT	Transcription regulator activity	7	1.4E−5	6.2E−4
GOTERM_BP_FAT	Regulation of RNA metabolic process	7	2.0E−5	5.2E−3
GOTERM_MF_FAT	Transcription factor activity	6	3.0E−5	6.5E−4
GOTERM_BP_FAT	Regulation of transcription	7	1.2E−4	1.5E−2
GOTERM_MF_FAT	DNA binding	7	1.2E−4	1.8E−3
GOTERM_BP_FAT	Regulation of transcription, DNA-dependent	6	3.6E−4	3.0E−2
SP_PIR_KEYWORDS	Nucleus	6	2.3E−3	5.9E−2
Annotation cluster 2	**Enrichment score: 2.06**	**Count**	**P value**	**Benjamini**
GOTERM_MF_FAT	Transcription factor activity	6	3.0E−5	6.5E−4
SP_PIR_KEYWORDS	Nucleus	6	2.3E−3	5.9E−2
GOTERM_MF_FAT	Transcription activator activity	3	4.7E−3	4.9E−2
SP_PIR_KEYWORDS	Transcription regulation	4	5.0E−3	6.3E−2
SP_PIR_KEYWORDS	Transcription	4	7.6E−3	4.9E−2
GOTERM_BP_FAT	Positive regulation of transcription	3	1.4E−2	5.9E−1
GOTERM_BP_FAT	Positive regulation of gene expression	3	1.5E−2	5.4E−1
GOTERM_BP_FAT	Positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	3	1.7E−2	5.2E−1
GOTERM_BP_FAT	Positive regulation of nitrogen compound metabolic process	3	1.8E−2	4.8E−1
GOTERM_BP_FAT	Positive regulation of macromolecule biosynthetic process	3	2.0E−2	4.7E−1
GOTERM_BP_FAT	Positive regulation of cellular biosynthetic process	3	2.1E−2	4.2E−1
GOTERM_BP_FAT	Positive regulation of biosynthetic process	3	2.2E−2	4.0E−1
GOTERM_BP_FAT	Positive regulation of macromolecule metabolic process	3	2.9E−2	4.7E−1
GOTERM_BP_FAT	Transcription	3	7.0E−2	7.6E−1
Annotation cluster 3	**Enrichment score: 1.61**	**Count**	**P value**	**Benjamini**
GOTERM_CC_FAT	Nucleoplasm	3	4.3E−3	2.3E−1
GOTERM_CC_FAT	Nuclear lumen	3	1.1E−2	2.8E−1
GOTERM_CC_FAT	Intracellular organelle lumen	3	2.0E−2	3.3E−1
GOTERM_CC_FAT	Organelle lumen	3	2.0E−2	2.6E−1
GOTERM_CC_FAT	Membrane-enclosed lumen	3	2.1E−2	2.3E−1
SP_PIR_KEYWORDS	Phosphoprotein	3	6.2E−1	9.9E−1

Gene functional classification of the 28 up-regulated TF genes (fold change ≤ 2.13–39.1) of hENSCs revealed the presence of two groups: the first group with an enrichment score of 10.22 contained 8 different TF functional groups, and the second group with an enrichment score of 8.58 contained 12 different TF functional groups. (Table 2).

3.7. GO clustering of up-regulated TF genes of hENSCs

GO clustering using DAVID for the 28 up-regulated TF genes (fold change ≤ 2.13–39.1) of hENSCs revealed 11 clusters (Table 2). The first annotation cluster with an enrichment score of 25.33 include: transcription factor activity (28 genes), transcription regulator activity (28 genes), and DNA binding (28 genes). The second annotation cluster with an enrichment score of 13.25 include: regulation of transcription, DNA-dependent (22 genes), transcription regulation (21 genes), regulation of RNA metabolic process (22 genes), transcription (21 genes), regulation of transcription (24 genes), transcription (22 genes), and nucleus (24 genes).

3.8. KEGG pathway analysis of TF up-regulated genes of hENSCs

KEGG pathway analysis of the 28 TF up-regulated genes (fold change ≤ 2.13–39.1) of hENSCs showed significant enrichment for pathways in cancer, Notch signaling pathway, and chronic myeloid leukemia (Table 3).

3.9. TF genes relevant to proliferation

The differences in the neuronal and glial differentiation potential between hENSC and OBNSC that had been revealed in the present study could be explained based on their TF gene expression profile. In this respect, it had been demonstrated that ZHX2 was up-regulated in hENSC as compared to OBNSC. Blocking ZHX2 function in cultured neural progenitor cells or in the embryonic cortex causes neuronal differentiation, whereas overexpression of ZHX2 disrupts the normal differentiation of cortical neural progenitor cells [25]. The high expression level of ZHX2 in our hENSC might explain the relatively low neuronal differentiation potential (25% neurons) relevant to the higher glial differentiation (75% astrocytes) potential.

The comparatively higher neuronal differentiation potential of hENSCs in comparison to OBNSC that had been demonstrated in the present work might be further explained by the observation that HES6 was up-regulated in hENSCs relative to OBNSC where Hes6 is known to play a role in primary neurogenesis. Depletion of Hes6 by morpholino antisense oligonucleotides results in a failure of neural differentiation [26].

MSRB2, KDM5B, and HDACs were up-regulated in our hENSC as compared to OBNSc. The overexpression MSRB2 is known to preserve the mitochondrial integrity, and hence contribute to cell proliferation, survival and protein maintenance [27]. KDM5B activates self-renewal-associated gene expression [40]. HDACs negatively control histone acetylation and occupies mainly active genes, including important regulators of NSC self-renewal and proliferation [41].

FOXO4 was found to be down-regulated in hENSCs as compared to our adult OBNSC. Loss of FOXO function has been identified in several human cancers, and results in increased cellular survival and a predisposition to neoplasia, especially in epithelial cancer. FOXO factors are therefore bona fide tumor suppressors. The low constitutive expression level of FOXO4 in hENSCs in comparison to OBNSC might indicate their higher tumorigenic potential in comparison to OBNSCs. Among other TFs related to proliferation activities of NSC, Foxn2 was found to be up-regulated in our OBNSCs. In this respect Foxn2 had been identified as key regulators of embryogenesis, cell cycling, cell lineage restriction and cancer [28].

Another proliferation-related TF gene that was up-regulated in our OBNSC is TGIF1 which negatively affects TGFβ signaling, and is implicated in regulation of cell growth, apoptosis and differentiation. TGFβ has been implicated in various human disorders ranging from vascular diseases to cancer. Cancer cell lines with inactivating mutations in Fbxw7 show enhanced levels of TGIF1 and attenuated TGFβ-dependent regulation of cell growth and migration [29]. The up-regulation of TGIF1 TF genes in our OBNSCs might point to their enhanced growth,

Table 2
GO clustering using DAVID for the 28 up-regulated TF genes (fold change ≤2.13–39.1) of hENSCs.

Annotation cluster 1	Enrichment score: 25.33	Count	P value	Benjamini
GOTERM_MF_FAT	Transcription factor activity	28	3.1E−31	2.1E−29
GOTERM_MF_FAT	Transcription regulator activity	28	5.0E−26	1.7E−24
GOTERM_MF_FAT	DNA binding	28	6.4E−21	1.5E−19
Annotation cluster 2	**Enrichment score: 13.25**	**Count**	**P value**	**Benjamini**
GOTERM_BP_FAT	Regulation of transcription, DNA-dependent	22	9.0E−15	3.4E−12
SP_PIR_KEYWORDS	Transcription regulation	21	1.1E−14	7.1E−13
GOTERM_BP_FAT	Regulation of RNA metabolic process	22	1.4E−14	2.7E−12
SP_PIR_KEYWORDS	Transcription	21	1.7E−14	5.4E−13
GOTERM_BP_FAT	Regulation of transcription	24	4.4E−14	5.6E−12
GOTERM_BP_FAT	Transcription	22	2.8E−13	2.7E−11
SP_PIR_KEYWORDS	Nucleus	24	6.4E−12	1.4E−10
Annotation cluster 3	**Enrichment score: 6.89**	**Count**	**P value**	**Benjamini**
GOTERM_BP_FAT	Regulation of transcription from RNA polymerase II promoter	13	2.6E−9	1.9E−7
GOTERM_BP_FAT	Negative regulation of transcription, DNA-dependent	10	1.2E−8	7.2E−7
GOTERM_BP_FAT	Negative regulation of RNA metabolic process	10	1.3E−8	7.2E−7
SP_PIR_KEYWORDS	Repressor	9	9.8E−8	1.3E−6
GOTERM_BP_FAT	Negative regulation of transcription	10	1.0E−7	4.8E−6
GOTERM_BP_FAT	Negative regulation of gene expression	10	2.3E−7	9.5E−6
GOTERM_BP_FAT	Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	10	2.6E−7	9.8E−6
GOTERM_BP_FAT	Negative regulation of nitrogen compound metabolic process	10	2.9E−7	1.0E−5
GOTERM_BP_FAT	Negative regulation of macromolecule biosynthetic process	10	4.6E−7	1.4E−5
GOTERM_BP_FAT	Negative regulation of macromolecule metabolic process	11	5.0E−7	1.4E−5
GOTERM_BP_FAT	Negative regulation of transcription from RNA polymerase II promoter	8	5.0E−7	1.3E−5
GOTERM_BP_FAT	Negative regulation of cellular biosynthetic process	10	5.6E−7	1.4E−5
GOTERM_BP_FAT	Negative regulation of biosynthetic process	10	6.7E−7	1.6E−5

Table 3
KEGG pathway analysis of the 28 TF up-regulated genes (fold change ≤2.13–39.1) of hENSCs showed significant enrichment for three pathways (pathways in cancer, Notch signaling pathway, and chronic myeloid leukemia).

Category	Term	Count	%	P-value	Benjamini
KEGG_PATHWAY	Pathways in cancer	3	1.1	5.2E−2	7.1E−1
KEGG_PATHWAY	Notch signaling pathway	2	0.7	5.4E−2	4.7E−1
KEGG_PATHWAY	Chronic myeloid leukemia	2	0.7	8.5E−2	5.0E−1

proliferation, and differentiation potentials. HNRNPK, a transcriptional coactivator of p53 was up-regulated in both our OBNSCs and hENSC. Phosphorylation of HNRNPK by Aurora-A (AURKA) disrupts its interaction with p53. Inverse correlation between Aurora-A (AURKA) activity and HNRNPK–p53 interaction was demonstrated in DNA-damaged cells [30]. Among the 28 TF genes that were up-regulated (fold change ≤2.13–39.1) (Fig. 3, Tables S1, S2) in hENSCs, several TFs were found to be related to the proliferation control. In this respect, in comparison to our OBNSCs, TRIM25, and Hoxc8 were up-regulated in hENSC and both are known to be involved in various cellular processes, including cell proliferation activity [31]. Moreover, Hoxc8 reduces cell adhesion and concomitant cell migration [32]. Whether or not the high expression level of Hoxc8 identified in our hENSC could compromise their migration and differentiation potential in comparison to OBNSC is a matter that still needs more elucidation.

3.10. Tumorigenic potential of hENSC vs. OBNSC

Next, we had assessed the tumorigenic potential of hENSC and OBNSC based on their TF gene expression profile. Tumor suppression TF genes such as CTNNB1 were also up-regulated in OBNSC. Defects in CTNNB1 are a cause of medulloblastoma [33]. CTNNB1 is a central component of the Wnt/β-catenin signaling pathway which plays an important role in adult neurogenesis [33]. The high expression profile of CTNNB1 in our OBNSCs might highlight the crucial role of OBNSCs in the generation of new neurons during adult life.

HDAC2 is up-regulated in hENSCs, a finding that might indicate its pronounced tumorigenic potential as compared to the adult OBNSCs. Histone deacetylases (HDAC2) control cellular signaling and gene expression. It is crucial for embryonic development, affects cytokine signaling relevant for immune responses and is often significantly over-expressed in solid tumors [34]. ATF4 was up-regulated in our hENSCs. ATF4 mutations is found in tumor cells and associated with anticancer drug resistance [35]. Such finding might indicate the possible tumorigenic potential of our hENSC in comparison to the OBNSCs.

Other tumorigenic TF genes such as YEATS, YEATS4, HOPX, ETV5 were also found to be up-regulated in hENSC as compared to OBNSC. In this regard, YEATS domain family members, such as YEATS4, MLLT1, and MLLT3, were found to have a strong link to cancer. YEATS4 is amplified in glioblastomas and astrocytomas; MLLT1 and MLLT3 are among the most frequent translocation partners of the mixed lineage leukemia (MLL) gene [36]. The high expression level of YEATS in our hENSC might highlight the possible tumorigenic potential of these cells following engraftments as compared to OBNSCs. Transcriptional silencing of HOPX results from hypermethylation of the HOP promoter leads to cancer development [37]. ETV5 is central to both tumor invasion and angiogenesis [38]. The up-regulation of ETV5 in our hENSC might highlight the possible tumorigenic potential of this cell line. HOXB7 acts as a key regulator, orchestrating a major group of target molecules in the oncogenic hierarchy. Higher expression levels of HOXB7 in the tumor significantly correlated with poorer disease-free survival in ERα-positive patients with breast cancer on adjuvant tamoxifen monotherapy [39].

Relevant to the tumorigenic potential of human OBNSC, it has been previously reported that the tumor-like growth properties of the stem cells associate with changes either in oncosuppressors or in oncogenes [40]. Tumorigenic olfactory bulb NS/PCs showed up-regulation of genes related to cell proliferation and inhibiting apoptosis, though solely hTERT and NOTCH1 were overexpressed independently from mitogen stimulation. Tumorigenic OBNSCs did express the hTERT protein, which was undetectable in non-tumorigenic NS/PCs, consistent with that reported in normal NS cells [27]. Immunofluorescence with anti-NOTCH1 antibody on tumorigenic OBNSCs demonstrated either increased cytoplasmic staining or abnormal nuclear staining. Following NOTCH1 blockade with the γ-secretase inhibitor X (GSI), OBNSCs lost their ability to form soft-agar colonies suggesting a functional role of

NOTCH1 in the tumorigenicity of these cells. Although the xenografts grown after injection of OB3a and SS-OB2a NS/PCs were histologically reminiscent of different tumors, in both of them molecular analyses pointed to hTERT and NOTCH1 as critical pathways. Telomerase is highly expressed in the majority of human cancers including glioblastoma, where it is believed to contribute to tumor progression because telomerase-dependent telomere maintenance provides cells with an extended proliferative potential [27]. NOTCH is known to promote the proliferation of nonneoplastic NSCs and to inhibit their differentiation; it is also highly activated in embryonal brain tumors, such as medulloblastoma, where it is required both for maintaining the stem cell fraction *in vitro* and for tumor formation *in vivo* [41]. Up-regulation of hTERT and NOTCH1 in tumorigenic OBNSCS suggests that a common mechanism may underlay the malignant transformation of these cells [27].

3.11. Epigenetic control

Differences in the proliferation and differentiation potentials between the embryonic and adult NSC during *in vitro* condition might be explained based on variation in the genomic and epigenetic profiles of the two cell populations. In this regard, Marei et al. [11,12] used the transcriptional signature of hENSC and adult human OBNSCs to decipher genes involved in epigenetic modifications such as SMARCC1, SMARCE1, and DNMT3B which were found specifically up-regulated in our OBNSC but not in hENSC and may interact with proteins encoded by other specific genes such as ARID2 and ARID1B [42]. Another TF that is relevant to epigenetic control of NSC is TAF12 which was up-regulated in OBNSCs in comparison to our hENSCs. The TAF12 is a TBP-associated factor that is contained in Pol I- and Pol II-specific TBP–TAF complexes, and it plays a crucial role in demethylation of rDNA [43].

4. Conclusion

Changes in expression of the cytoplasmic markers and transcription factor genes associated with neurogenesis, proliferation and differentiation in human embryonic neural stem cells (hENSCs), and adult human olfactory bulb neural stem cells (OBNSCs) were examined by immunohistochemistry and DNA microarray. Eleven, and 28, TF genes were up-regulated in OBNSCs, and hENSC, respectively. GO clustering and KEGG pathway analysis revealed a significant enrichment for the basal transcription factor pathway, and Notch signaling pathway in OBNSC, and hENSC respectively.

Immunofluorescence analysis of the differentiated hENSC and OBNSC revealed a significantly greater number of β-tubulin III (TUBB3), MAP, glial fibrillary acidic protein (GFAP), and O4 in hENSC when compared to those in OBNSC. Furthermore, the expression of epigenetic-related TF-genes SMARCC1, TAF12, and UHRF1 increased significantly in OBNSC when compared with hENSC. Taken together, the present study reveals distinct proliferation, differentiation and epigenetic control between hENSC and OBNSC. Such finding is expected to generate new advances for selecting the best cell source for future treatments for different traumatic and neurodegenerative CNS injuries.

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References

[1] M.A. Curtis, M. Kam, U. Nannmark, M.F. Anderson, M.Z. Axell, C. Willelso, et al., Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension, Science 315 (2007) 1243–1249.
[2] A. Villa, F.J. Rubio, B. Navarro, C. Bueno, A. Martínez-Serrano, Human neural stem cells *in vitro*. A focus on their isolation and perpetuation, Biomed. Pharmacother. 55 (2001) 91–95.
[3] A.L. Vescovi, E.Y. Snyder, Establishment and properties of neural stem cell clones: plasticity *in vitro* and *in vivo*, Brain Pathol. 70 (1999) 569–598.
[4] Z. Liu, L.J. Martin, Olfactory bulb core is a rich source of neural progenitor and stem cells in the adult rodent and human, J. Comp. Neurol. 459 (2003) 368–391.
[5] P. Casalbore, M. Budoni, L. Ricci-Vitiani, C. Cenciarelli, G. Petrucci, Tumorigenic potential of olfactory bulb-derived human adult neural stem cells associates with activation of TERT and NOTCH1, PLoS One 4 (2) (2009) e4434.
[6] A.P. Lee, Y. Yang, S. Brenner, B. Venkatesh, TFCONES: a database of vertebrate transcription factor-encoding genes and their associated conserved noncoding elements, BMC Genomics 8 (2007) 441.
[7] J.D. Cahoy, et al., A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function, J. Neurosci. 28 (2008) 264–278.
[8] L. Lo, L. Sommer, D.J. Anderson, MASH1 maintains competence for BMP2-induced neuronal differentiation in post-migratory neural crest cells, Curr. Biol. 7 (1997) 440–450.
[9] M. Gotz, Making glutamatergic neurons from GABAergic progenitors, Nat. Neurosci. 13 (2010) 1308–1309.
[10] K. Takahashi, et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[11] H.E.S. Marei, A. Althani, N. Afifi, F. Michetti, M. Pescatori, et al., Gene expression profiling of embryonic human neural stem cells and dopaminergic neurons from adult human substantia nigra, PLoS One 6 (12) (2011) e28420, http://dx.doi.org/10.1371/journal.pone.0028420.
[12] H.E.S. Marei, A.-E. Ahmed, F. Michetti, M. Pescatori, R. Pallini, et al., Gene expression profile of adult human olfactory bulb and embryonic neural stem cell suggests distinct signaling pathways and epigenetic control, PLoS One 7 (4) (2012) e33542, http://dx.doi.org/10.1371/journal.pone.0033542.
[13] B.A. Reynolds, S. Weiss, Clonal and population analyses demonstrate that an EGF responsive mammalian embryonic CNS precursor is a stem cell, Dev. Biol. 175 (1996) 1–13.
[14] P. Casalbore, M. Budoni, L. Ricci-Vitiani, C. Cenciarelli, G. Petrucci, et al., Tumorigenic potential of olfactory bulb-derived human adult neural stem cells associates with activation of TERT and NOTCH1, PLoS One 4 (2) (2009) e4434.
[15] P. Du, W.A. Kibbe, S.M. Lin, Illumina pipeline for processing Illumina microarray, Bioinformatics 24 (2008) 1547–1548.
[16] P. Pavlidis, J. Qin, V. Arango, J.J. Mann, E. Sibille, Using the Gene Ontology for microarray data mining: a comparison of methods and application to age effects in human prefrontal cortex, Neurochem. Res. 29 (2004) 1213–1222.
[17] Y. Benjamini, D. Drai, G. Elmer, N. Kafkafi, I. Golani, Controlling the false discovery rate in behavior genetics research, Behav. Brain Res. 125 (2001) 279–284.
[18] R.A. Irizarry, B. Hobbs, F. Collin, Exploration, normalization, and summaries of high density oligonucleotide array probe level data, Biostatistics 4 (2003) 249–264.
[19] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, Gene Ontology: tool for the unification of biology, Nat. Genet. 25 (2000) 25–29.
[20] M. Kanehisa, S. Goto, M. Hattori, K.F. Aoki-Kinoshita, M. Itoh, S. Kawashima, T. Katayama, M. Araki, M. Hirakawa, From genomics to chemical genomics: new developments in KEGG, Nucleic Acids Res. 34 (2006) D354–D357.
[21] G. Joshi-Tope, M. Gillespie, I. Va¨ strik, P. D'Eustachio, E. Schmidt, B. de Bono, B. Jassal, G.R. Gopinath, G.R. Wu, L. Matthews, Reactome: a knowledgebase of biological pathways, Nucleic Acids Res. 33 (2005) D428–D432.
[22] V. Matys, O.V. Kel-Margoulis, E. Fricke, I. Liebich, S. Land, A. Barre-Dirrie, I. Reuter, D. Chekmenev, M. Krull, K. Hornischer, TRANSFAC and its module TRANSCompel: transcriptional gene regulation in eukaryotes, Nucleic Acids Res. 34 (2006) D108.
[23] S. Wang, M.S. Okun, O. Suslov, et al., Neurogenic potential of progenitor cells isolated from postmortem human Parkinsonian brains, Brain Res. 29 (1464) (2012) 61–72.
[24] I.S. Shimada, M.D. Lecomte, J.C. Granger, N.J. Quinlan, J.L. Spees, Self-renewal and differentiation of reactive astrocyte-derived neural stem/progenitor cells isolated from the cortical peri-infarct area after stroke, J. Neurosci. 6 (32(23)) (2012) 7926–7940.
[25] C. Wu, R. Qiu, J. Wang, H. Zhang, K. Murai, Q. Lu, ZHX2 interacts with Ephrin-B and regulates neural progenitor maintenance in the developing cerebral cortex, J. Neurosci. 10 (29(23)) (2009) 7404–7412.
[26] K. Murai, A. Philpott, P.H. Jones, Hes6 is required for the neurogenic activity of neurogenin and NeuroD, PLoS One 6 (11) (2011) e27880.
[27] F. Cabreiro, C.R. Picot, M. Perichon, J. Castel, B. Friguet, I. Petropoulos, Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage, J. Biol. Chem. 13 (283(24)) (2008) 16673–16681.
[28] C. Tribioli, R.F. Robledo, T. Lufkin, The murine fork head gene Foxn2 is expressed in craniofacial, limb, CNS and somitic tissues during embryogenesis, Mech. Dev. 118 (1–2) (2002) 161–163.
[29] M.T. Bengoechea-Alonso, J. Ericsson, Tumor suppressor Fbxw7 regulates TGFβ signaling by targeting TGIF1 for degradation, Oncogene 29 (38) (2010) 5322–5328.
[30] K.W. Hsueh, S.L. Fu, C.Y. Huang, C.H. Lin, Aurora-A (AURKA) phosphorylates hnRNPK and disrupts its interaction with p53, FEBS Lett. 2 (585(17)) (2011) 2671–2675.
[31] Michaela U. Gack, Young C. Shin, Chul-Hyun Joo1, Tomohiko Urano, Chengyu Liang, Lijun Sun, Osamu Takeuchi, Shizuo Akira, Zhijian Chen, Satoshi Inoue, Jae U. Jung, TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity, Nature 446 (2007) 916–920.
[32] K. Ruthala, J. Gadi, J.Y. Lee, H. Yoon, H.J. Chung, M.H. Kim, Hoxc8 downregulates Mgl1 tumor suppressor gene expression and reduces its concomitant function on cell adhesion, Mol. Cells 32 (3) (2011) 273–279.

[33] L. Zhang, X. Yang, S. Yang, J. Zhang, The Wnt/β-catenin signaling pathway in the adult neurogenesis, Eur. J. Neurosci. 33 (1) (2011) 1–8.
[34] O.H. Krämer, HDAC2: a critical factor in health and disease, Trends Pharmacol. Sci. 30 (12) (2009) 647–655.
[35] J. Lewerenz, H. Sato, P. Albrecht, N. Henke, R. Noack, A. Methner, P. Maher, Mutation of ATF4 mediates resistance of neuronal cell lines against oxidative stress by inducing xCT expression, Cell Death Differ. 19 (5) (2012) 847–858.
[36] J.M. Schulze, A.Y. Wang, M.S. Kobor, YEATS domain proteins: a diverse family with many links to chromatin modification and transcription, Biochem. Cell Biol. 87 (1) (2009) 65–75.
[37] S. Yamaguchi, K. Asanoma, T. Takao, K. Kato, N. Wake, Homeobox gene HOPX is epigenetically silenced in human uterine endometrial cancer and suppresses estrogen-stimulated proliferation of cancer cells by inhibiting serum response factor, Int. J. Cancer 1 (124(11)) (2009) 2577–2588.
[38] M. Llauradó, M. Abal, J. Castellví, S. Cabrera, A. Gil-Moreno, A. Pérez-Benavente, E. Colás, A. Doll, X. Dolcet, X. Matias-Guiu, M. Vazquez-Levin, J. Reventós, A. Ruiz, ETV5 transcription factor is overexpressed in ovarian cancer and regulates cell adhesion in ovarian cancer cells, Int. J. Cancer 130 (7) (2012) 1532–1543.
[39] K. Jin, X. Kong, T. Shah, M.F. Penet, F. Wildes, D.C. Sgroi, X.J. Ma, Y. Huang, A. Kallioniemi, G. Landberg, I. Bieche, X. Wu, P.E. Lobie, N.E. Davidson, Z.M. Bhujwalla, T. Zhu, S. Sukumar, The HOXB7 protein renders breast cancer cells resistant to tamoxifen through activation of the EGFR pathway, Proc. Natl. Acad. Sci. U. S. A. 109 (8) (2012) 2736–2741.
[40] G. Pichler, P. Wolf, C.S. Schmidt, D. Meilinger, K. Schneider, C. Frauer, K. Fellinger, A. Rottach, H. Leonhardt, Cooperative DNA and histone binding by Uhrf2 links the two major repressive epigenetic pathways, J. Cell. Biochem. 112 (9) (2011) 2585–2593.
[41] X. Fan, W. Matsui, L. Khaki, D. Stearns, J. Chun, et al., Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors, Cancer Res. 66 (2006) 7445–7452.
[42] M. Nemir, A. Croquelois, T. Pedrazzini, F. Radtke, Induction of cardiogenesis in embryonic stem cells via downregulation of Notch1 signaling, Circ. Res. 98 (2006) 1471–1478.
[43] F.B. Briggs, B.A. Goldstein, J.L. McCauley, R.L. Zuvich, P.L. De Jager, J.D. Rioux, A.J. Ivinson, A. Compston, D.A. Hafler, S.L. Hauser, J.R. Oksenberg, S.J. Sawcer, M.A. Pericak-Vance, J.L. Haines, L.F. Barcellos, Variation within DNA repair pathway genes and risk of multiple sclerosis, Am. J. Epidemiol. 15 (172(2)) (2010) 217–224.