Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways

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ABSTRACT
Human embryonic stem cells can be differentiated along different lineages, providing the possibility of a precise analysis of genes profiles associated with specific commitments. Subtractive gene expression profiling between differentiated and undifferentiated cells provides lists of potential actors in this commitment. This combines, however, genes that are specifically associated with development and others that are over expressed because of non-lineage-specific differentiation systems. As a way to establish gene profiles associated with the neural and/or to the mesodermal commitments of human embryonic stem cells more precisely, we have carried out a two-step analysis. We first performed a subtractive analysis of gene profiles of each of these lineages as compared to the undifferentiated stage. Then, we extended the analysis by comparing the two sets of results with each other. This strategy has allowed us to eliminate large numbers of genes that were over expressed in both sets of results and to uniquely associate different gene networks with either the neural or the mesodermal commitments.

INTRODUCTION
For one decade, human embryonic stem cells (hES) have been recognized as a valuable model for studies of early steps of human development since the acquisition of a defined phenotype in vitro follows sequential activation of gene networks and epigenetic changes that closely mimic events occurring in vivo during embryogenesis [1-5]. These neuroectodermic clusters of cells are readily induced in neural progenitor cells (NPC) to directly visualize the process of neural conversion by forming of columnar epithelial cells of NPCs with defined neural cell lineages exhibiting neural inducing activity, it is possible to differentiate NPCs into neural progenitor cells for neural development [6,7]. Nevertheless, analysis of how genes are controlled during transition toward a dedicated developmental path is restricted mainly by the ability to obtain homogeneous populations of cells because their phenotypes are continuously changing over time. Several studies clearly demonstrate that such approaches applied to hES and their progenies can provide useful information about development processes and control of developmentally important processes for neural cell lineages [8-11]. An example of such approaches is the report on the generation of neural rosettes as columnar epithelial cells radially organized resembling a cross section of neural tube [10,11].

Over the last few years, important efforts focused on the possibility of controlling the differentiation of hES particularly for neural fate. Indeed, using protocols based on the co-culture of hES with defined stromal cell lines exhibiting neural inducing activity, it is possible to directly visualize the process of neural conversion by forming of columnar epithelial cells of hES, exhibiting neural inducing activity, based on the co-culture of hES with defined stromal cell lines exhibiting neural inducing activity. This system of culture is considered to be relevant for in vitro modeling of neural development. Nevertheless, methods used and the protocols can provide useful information about development processes and control of developmentally important processes for neural cell lineages [8-11]. An example of such approaches is the report on the generation of neural rosettes as columnar epithelial cells radially organized resembling a cross section of neural tube [10,11].

Given the importance of understanding how genes are controlled during transition toward a dedicated developmental path, alternative approaches have been developed to control the differentiation of hES, particularly for neural fate. These approaches include the use of defined stromal cell lines exhibiting neural inducing activity, allowing for the direct visualization of the process of neural conversion by forming of columnar epithelial cells of hES, exhibiting neural inducing activity, based on the co-culture of hES with defined stromal cell lines exhibiting neural inducing activity. This system of culture is considered to be relevant for in vitro modeling of neural development. Nevertheless, methods used and the protocols can provide useful information about development processes and control of developmentally important processes for neural cell lineages [8-11]. An example of such approaches is the report on the generation of neural rosettes as columnar epithelial cells radially organized resembling a cross section of neural tube [10,11].

Human embryonic stem cells have been used to study alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331) that reveal alternative developmental pathways (doi: 10.1089/scd.2010.0331). This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.
probably due to no synchronized processes [15]. Consequently, neural rosettes represent structures that contain a mixture of cells, including neuroepithelial progenitor cells (NEPC), neural stem cells (NSC), committed neurons at different stages of their development and probably neural crest derivatives at their periphery. For this reason, several groups have attempted to develop cell selection strategies including the use of fluorescence-activated cell sorting (FACS). To date, only few membrane markers have been used to enrich cultures in neural precursors and neurons [16]. Among these markers, we used Neural Cell Adhesion Molecule (NCAM/CD56) in this work [17].

The production of mesenchymal precursors (MPC) has not been as widely reported as that of neural precursors. By taking advantages of the recent development of protocols triggering the differentiation of hES toward a near-homogenous amplifiable population of mesenchymal progenitors exhibiting a phenotype of Mesenchymal Stem Cells-like (MSC) can be obtained [18-23]. In this study, we produced highly homogenous cell populations for both neural and mesenchymal precursors by engagement of the hES cells into either the neural or the mesodermal lineages. The analysis of gene expression patterns of these two populations, sharing the same genetic background, compared to the same starting population that were hES cells, using strictly identical procedures for hybridization and statistical analysis, allowed us to select genes that were modulated in opposite directions during commitment to either neural or mesenchymal fates. After this subtractive analysis, selected genes exhibiting modulations specific for either neural or mesenchymal precursors were used to build in silico global gene networks and, using a comparative strategy, to determine their implications as actors in the main signalling pathways involved in early steps of human development.

MATERIALS AND METHODS
**Human ES Cells Culture**

Two hES cell lines, SA01, (XY, passage 40, Cellartis, Sweden) and VUB01 (XY, passage 80, AZ-VUB, Belgium) were maintained and propagated on a feeder layer of STO (SIM mice Thioguanine and Quabaine resistant) murine embryonic fibroblast cells inactivated by Mitomycin C (Sigma Aldrich, 2.5 µg per mL overnight at 37°C). Cells were cultured in a humidified 5% CO2 incubator at 37°C in a serum replacement medium (Knock-out DMEM, 20% Knock-out Serum Remplacement (KSR), 1% Glutamax 1mM, 1% Non Essential Amino Acid (NEAA), 0.1% Beta-Mercaptoethanol (BM) 0.1% and 1% Penicillin/streptomycin (P/S), all from GIBCO) supplemented with 8ng/mL of bFGF (Invitrogen). The culture media was changed daily and routine passages routinely performed by mechanical cutting of hES cells on a fresh feeder layer every 4-5 days.

**Differentiation of hES cells toward NPC, neurons and astrocytes**

**Differentiation of hES cells toward NPC**

The differentiation of hES toward neurectodermal rosettes was adapted from the Stromal Differentiation Inducing Activity protocol as described elsewhere [10]. Briefly, hES cells were manually dissociated from the STO feeder layer and plated at a density of approximately $10^3$ cells per cm² on a confluent layer of mitotically inactivated murine stromal feeder cells (MS5). Cells were cultured in KSR medium (Knock-out DMEM, 15% KSR; 1% Glutamax; 1% NEAA and 0.1% BM, all from GIBCO) for 14-16 DIV (Days In Vitro), when the medium was replaced by Neurobasal medium, N2 (DMEM-F12+ Glutamax, 1% N2 supplement and 1% P/S) until DIV21.

**Cell sorting**

Cells were harvested at DIV21 using TrypLE Express (GIBCO) and about $5.10^6$ cells were suspended in PBS-2% Fetal Calf Serum containing 1% 7-amino-actinomycin D (7AAD)
(Sigma) and then incubated with IgG1κ Direct conjugated Phyco-erythrin (PE) monoclonal anti human Neural Cell Adhesion Molecule (hNCAM) antibody diluted 1/10 provided by BD Biosciences Pharmingen™. This antibody recognizes an extracellular immunoglobulin-like domain common to three molecular weight forms –Mw 120, 140 and 180 kilodaltons –of the NCAM protein. The cell sorting was performed by a MoFlow Cell Sorter Cytometer from Cytomation and positive and negative fractions were collected in 1mL of N2 medium with 1% P/S.

**Differentiation of NPC toward neurons and astrocytes**

After sorting, the NCAM+ cells were seeded on Poly-Orynthine (15µg/mL,Gibco)/laminin (1mg/mL,Sigma) coated dishes (50x10³ /cm²) in N2 medium supplemented with growth factors bFGF (20ng/ml, Invitrogen), and EGF (10ng/ml, Abcys) to allow their proliferation for 10-15 days. The medium was changed every two days. When confluent, they were passaged (P1) after exposure to collagenase 1mg/ml for 15 minutes at 37°C and plated in N2 medium supplemented for differentiation toward either neurons or astrocytes. For neuronal differentiation, BDNF (10 ng/mL) and AA ascorbic acid (10 ng/mL) was added in the N2 medium which was changed every 2-3 days for 2 weeks after which the cells were fixed. For differentiation toward astrocytes, medium was supplemented with EGF and CNTF (20ng/ml, R&D) for 15 days. After that, they were passaged and maintained in N2 medium containing only CNTF until around 100 DIV and then fixed for immunochemistry.

**Differentiation of hES cells toward MPC cells, osteoblasts and adipocytes**

_Differentiation of hES cells toward MPC cells_
Mesodermal differentiation was obtained as previously described by our laboratory [22] using a modified protocol from Barberi et al [23]. Briefly, differentiation was induced by plating $2 \times 10^4$ ES cells/cm$^2$ on 0.1% gelatin coated dishes in the presence of KO-DMEM medium supplemented with 20% Fetal Bovine Serum (FBS, Invitrogen), 1mM L-glutamine, 1% NEAA, 1% P/S and 0.1mM BM. Medium was changed every 3 days. Confluent cells were passed with trypsin/EDTA 1X (Invitrogen) in new gelatine coated dishes. Immunophenotyping was carried out using a FACScalibur and the Cell Quest software (Becton&Dickinson Biosciences). More than 10,000 events were acquired for each sample and analysed. Cells were harvested as previously described and incubated for 30 minutes at room temperature with one of the following anti-human antibodies: CD73-PE (SH3/NT5E), CD44-PE, CD54-PE (I-CAM-1), CD29-PE (integrin $\beta$1), CD106-PE (VCAM), CD166-PE (ALCAM), CD14-PE, CD31-PE (PECAM-1), CD56-PE (NCAM), HLA-ABC-PE, HLA-DR-PE, CD34-APC, CD45-FITC (all from Becton&Dickinson Biosciences/Pharmingen); CD105-PE (SH2/Endoglin; Caltag); Nestin-PE (R&D systems) and primary monoclonal antibody vimentin and Stro1 were used with mouse IgG- or IgM-Alexa as secondary antibody. Mouse isotype antibodies served as respective controls (Becton&Dickinson). The acquisition was performed by the FACScalibur cytometer and data were analyzed with Cell Quest Pro Software (Beckton-Dikinson).

**Differentiation of MPC cells toward osteoblasts and adipocytes**

To induce osteoblastic differentiation, cells were plated at a density of 30 000 cells/cm$^2$ in a specific medium (Cambrex), containing dexamethasone, ascorbate and B-glycerophosphate (Sigma-Aldrich). After 21 days, cell phenotype was analysed by alkaline phosphatase activity (Sigma-Aldrich). Adipogenic differentiation was induced by culturing the cells in the specific medium (Cambrex) supplemented with 100µM linoleic acid. Adipogenesis was detected by the presence of neutral lipids in the cytoplasm stained with Oil Red O.
**Immunocytochemistry**

Cells were fixed in paraformaldehyde for 20 minutes at room temperature, rinsed with PBS and blocked with 1% BSA, 5% goat serum 0.1% triton in PBS solution for 1 hour and thereafter were incubated with the appropriate primary antibodies overnight at 4°C. Rabbit polyclonal antibodies included Nestin (dilution 1/500; Chemicon) and GFAP (dilution 1/1000; DAKO). Mouse Monoclonal antibodies (IgG) included Tuj1 (dilution 1/500, Covance), Stro-1 (dilution 1:100) and SMA (alpha smooth muscle: 1:100, DAKO). Appropriate Alexa 488 and Alexa 555 labeled secondary antibodies were used at 1/500 and 4’,6-diamino-2-phenylindole at 2µg/ml (Sigma) for counterstaining.

The preparations were analyzed by epifluorescence microscopy (Zeiss Imager Z1 and Zeiss Axiovert 40CFL) and images were captured with Axiocam mRM (Zeiss).

**RNA Sample preparation**

mRNA samples were extracted using the RNeasy Mini kit (Qiagen) according to the manufacturing protocols for undifferentiated hES cells, NPC and MPC. RNA samples were quantified using the Nanodrop photometer and quality controls were performed on BioAnalyzer 2100 (Agilent). For RT-PCR analysis, cDNAs were prepared by reverse transcription of 500 ng of mRNA using the SuperScript II Reverse Transcriptase kit according to the manufacturer’s instructions (Invitrogen). Primers used in this study are shown in Supplementary Table S1. Quantitative RT-PCR analyses were performed with Chromo4 Analyser (Biorad) and calculations were performed using the delta-delta C(t) method.

**Hybridization and data analysis**

RNA samples were labeled and hybridized on the GeneChip Human Genome HG_U133_Plus 2.0 Array (Affymetrix) by the RNG platform (Réseau National des Génopoles, Paris, France)
according to the Affymetrix procedures. Hybridization data were exploited using Array Assist 4.2 software (Stratagene). First, the software validated the quality controls. Next, the GC-RMA statistical algorithm procedure was used to normalize hybridization intensity values. A one way-ANOVA test was applied on transformed logarithm base 2 data to retain values that did not change significantly ($\alpha<0.05$) among triplicate samples. Identification of modulated genes was performed using the Student parametric statistical test adjusted with the FDR Benjamini-Hodgberg correction. A gene was considered as modulated for a Fold Change (FC) $>2$ with a corrected $p$-value, $\alpha_c<0.05$. The final list of modulated genes was established by removing duplicate data (multiple probesets measuring the same gene) to retain the most modulated one. GO biological processes and canonical pathways analysis enriched in differentially expressed genes were identified using the Fischer exact test as implemented in the Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com) software.

RESULTS

**Obtention of two homogenous neural and mesodermal progenitor cells from hES**

Two hES cell lines (SA_01 and VUB_01) were induced in triplicate toward the neural lineage using the SDIA protocol (see Material and Methods). Under these conditions, neural rosettes appeared around 16-18 days *in vitro* (DIV) (Supplementary Figure S1A). To get a homogenous population, cell sorting was performed at DIV21 using the membrane marker Neural Cell Adhesion molecule (NCAM/CD56) known to be expressed in neural precursors but not expressed in MS5 mouse feeder cells and to a small extent in the undifferentiated hES cells (Supplementary Figure S1B). The NCAM positive cells, termed NPC, expressed a combination of markers known to be specific for the neuroectodermal precursors such as NESTIN and the transcription factors \textit{SOX1}, \textit{PAX6} and \textit{OTX2}. Moreover, the NCAM positive cells exhibited a complete loss of \textit{NANOG} expression and did not express \textit{GFAP} (Glial
Fibrillary Acidic Protein) by RT-PCR (Supplementary Figure S1C) or by immunochemistry (Supplementary Figure S1 D-F). The NCAM positive cell population was able to give rise to cells positive for TUJ1 (βIII-tubulin) and for GFAP corresponding to neurons and astrocytes respectively (Supplementary Figure S1 G-H). Differentiation of ES cells into mesenchymal precursors (MPC) displaying a phenotype similar to that described by previous authors [18-21] was readily obtained after about 30 days of culture (two to three passages). Cells displayed a homogeneous fibroblast-like morphology (Supplementary Figure S2A). At near homogeneity, they expressed CD29 (β1-integrin), CD44 (H-CAM), CD73 (SH-3, ecto-5’-nucleotidase), CD105 (SH-2, endoglin), CD166 (ALCAM), and were negative for hematopoietic markers (CD34, CD45 and CD14), neuronal markers (NCAM/CD56 and FORSE1), and the endothelial marker CD31 (Supplementary S2B). Cells were immunoreactive for Stro1 and some of them are positive for α-SMA (alpha smooth muscle actin) (Supplementary Figure S2C). In addition, these MPC cells were also able to differentiate into osteogenic or adipogenic cells in appropriate conditions (Supplementary Figure S2D).

**Global analysis of gene expression**

To compare the expression patterns during the commitment of hES toward the neural and the mesenchymal fates, transcriptome analysis was performed in triplicate on the two undifferentiated hES cell lines SA01 and VUB01 and the two progenitor cell populations, NPC and MPC (Figure 1A). The global expression profiles of these three populations were compared by correlation plot and by the Principal Component Analysis (PCA). A high correlation coefficient was observed between the expression patterns of the three replicates for each cell line and between the two cell lines indicating a small variability between the biological samples for the same stage of differentiation (Figure 1B). Moreover, when the three
distinct populations were plotted by PCA, samples for the same stage were very close (Figure 1C). This allowed us to group the results obtained for the two cell lines for further statistical analysis. A total of 3167 genes were found to be differentially expressed between NPC and hES cells, 1727 up-regulated and 1440 down-regulated (Supplementary Tables S2 and S3), including genes with no annotation, putative genes and expressed sequence tags (EST) that amounted to about a quarter of all modulated genes. Parallel comparison between hES and MPC, revealed 5931 genes modulated including 2212 genes up-regulated and 3719 genes down-regulated in MPC (Supplementary Tables S4 and S5). As expected, major markers of pluripotent hES cells including NANOG; OCT4 [POU5F1]; REX1 [ZFP42]; FGF4; FOXD3; CLDN6; GDF3; DNMT3A and CD2 were down regulated in both NPC and MPC. However, the expression of the pluripotency transcription factor SOX2, was maintained in neural progenitors whereas it was switched off in mesenchymal progenitors.

As quality control of genes modulated in the transcriptomic experiment, we found specific neural genes expected to be up-regulated in NPC cells compared to hES, such as NCAM, PAX6, SOX1 and OTX2. On the contrary, neither markers of mesoderm such as T/Brachyury, HAND1, IGF2, CD45, FLK1, CD31, MYOD, CALP [Calponin] nor endodermal markers SOX17, FOXA2, GATA4, AFP were found modulated. In the same way, concerning genes known to be implicated in the MSC phenotype, CD73/NT5E, CD105/ENDOGLIN, CD44, INTEGRIN β1/ITGB1, ALCAM/CD166 and VIMENTIN, were all found to be up-regulated in MPC compared to hES.

**Subtractive gene expression profiling**
The lists of the modulated genes in the NPC and the MPC cell populations were compared (Figure 1D). The 785 genes which are up regulated in NPC but not in MPC (Supplementary Table S6) and the subset of 306 genes which were up regulated in NPC and down-regulated in MPC were selected as potential candidate genes involved in the neuralizing process (Supplementary Table S7). Conversely, the 1479 genes which are up-regulated in MPC but not in NPC (Supplementary Table S8) and the subset of 94 genes which were up-regulated in MPC and down-regulated in NPC were selected as potential candidate genes involved in the mesenchymal differentiation (Supplementary Table S9). Between the two selections of genes specifically implicated in the neuralization process or in the mesenchymal differentiation, we focused on transcription regulators which were specifically up-regulated in each of both processes (Table S10). For the neuralization process, among the 127 up-regulated transcription regulators (Table S10, left panel), for the eleven most modulated genes with a FC> 10, eight were annotated in nervous system development (GO and IPA classifications) including genes such as LHX2, PAX6, ZIC1, FOXG1B, TFAP2B, ZBTB16 and EMX2 all implicated in the neural progenitor signature. Besides these genes, others were annotated to be involved in neural and other developmental processes, such DACH1 and LEF1 or several members of family transcription factor such as the Inhibitor of DNA binding (ID2 and ID4), the POU domain family members (POU3F2 and POU4F1) but also the homeobox family members HOX (HOXA1 and HOXA9). In addition, genes found to be up-regulated in NPC and strongly down-regulated in MPC included SRY-related box protein members such as SOX3 and SOX11 or the member of the bicoïd sub-family of homeodomain-containing transcription factors such as OTX2 which encodes gene already reported to be involved in neurogenesis.

For the mesenchymal differentiation of the 118 up-regulated transcription regulators (Table S10, right panel), 25 were implicated in connective tissue development (IPA classification).
The most modulated genes were \textit{SIX1} which encodes of the homeobox gene superfamily and two genes related to TGF-beta signaling were highly up-regulated, \textit{CDKN2B} and \textit{TGF1B1I1}. Other modulated genes involved in development included several members of the Forkhead-box family (\textit{FOXD1}, \textit{FOXF1}, \textit{FOXF2}, \textit{FOXJ2}, \textit{FOXL1} and \textit{FOXP1}), the basic helix-loop-helix family (\textit{BHLHE40} and \textit{BHLHE4}), the Krüppel-like factors (\textit{KLF2}, \textit{KLF7} and \textit{KLF9}), signal transducers and activators of transcription (\textit{STAT1}, \textit{STAT2}, \textit{STAT3} and \textit{STAT6}), T-box members (\textit{TBX2} and \textit{TBX3}) and homeobox family members (\textit{HOXB2} and \textit{HOXB7}).

\textbf{Transcriptional networks using an \textit{in silico} approach}

Global gene networks were built based on selected genes exhibiting specific overexpression in each precursor, as described above, for either the neural (Figure 2A) or the mesenchymal differentiation (Figure 2B). Starting from the core pluripotency gene network composed of the three transcription factors, \textit{NANOG}, \textit{OCT4} and \textit{SOX2}, their potential targets were explored by selecting genes that were specifically up-regulated in each precursor cell population.

For the neural gene network, the starting point was the binding relationship linking \textit{PAX6} and \textit{LHX2} promoters with \textit{NANOG}, \textit{OCT4} and \textit{SOX2} proteins encoded by the core pluripotency genes. Using this strategy, the construction of the gene network step by step revealed some nodes that included key transcription factors including downstream \textit{NOTCH1} targets such as \textit{HES1}, \textit{HES5}, \textit{LEF1} but also \textit{PAX3} that may support a pivotal role with its complex partner \textit{SOX10}. Otherwise, \textit{NOTCH1} downstream signal was also connected with the SWI/SNF DNA remodelling complex (\textit{SMARCC4} and \textit{SMARCE1}).

For the mesenchymal gene network, \textit{WWTR1} (also termed TAZ) implicated downstream of \textit{TGFB1}, acted on the level of expression of \textit{NANOG} and \textit{POU5F1} and thus may control several developmental genes including \textit{GATA6}. Moreover, \textit{SOX2} (whose expression was maintained in neural but greatly decreased in mesenchymal differentiation) was connected with two critical genes involved in osteogenic differentiation, \textit{JUN} and \textit{TWIST1}. Organisation
of the network also included PPARG which is involved in differentiation of mesenchymal stem cells toward adipocytes and STAT3 which acts downstream of the FGF signalling pathway and is involved in differentiation process.

**Alternative signaling pathways controlling cell fate decisions**

Levels of gene expression were explored in three signaling pathways, Notch, Wnt, and TGFβ/Activin/BMP that are known to be involved in hES cell fates determination but required different partners depending on lineage-specific differentiation.

*Notch signaling pathway*

Some genes encoding for ligands of Notch, such as JAG1 and DLL1 were found to be up-regulated in the NPC but not in the MPC cells (Figure 3A and 3B). Regarding the receptors in this pathway, NOTCH1 was specifically up-regulated in the NPC cells, NOTCH2 was found up-regulated in both progenitor cell types whereas other family members of NOTCH receptors were not modulated. Genes implicated in the modulation of the activity of NOTCH signaling that were specifically up-regulated in NPC cells included: LFNG (encoded a fucose-specific glycosyltransferase), ADAM17 (encoded a metallopeptidase involved in the proteolytic release of Notch intracellular domain from the Notch1 receptor) and PSEN1 (presenilins-1) involved in the cleavage of the Notch receptor and the regulation of gamma secretase activity. On the contrary, NUMB encoding for an inhibitor of the Notch pathway and playing a role in the determination of cell fates during development was specifically over expressed in MPC. At least, specific transcriptional factors HES1 and HES5, downstream targets of Notch signaling were found specifically up-regulated in NPC cells but not in MPC whereas others family members were not modulated in either progenitor cell types. On the contrary, NUMB, encoding for an inhibitor of the Notch pathway and playing a role in the determination of cell fates during development. Additionally, others downstream
transcriptional factors of the Hairy/enhancer-of split related with YRPW motif family exhibited similar expression profiles in the two progenitor cell types (ie up-regulation of HEY1 and down regulation of HEY2). Specific over expression of DLL1, NOTCH1, HES1 and HES5 were confirmed in NPC by quantitative RT-PCR (Figure 3C).

Wnt Signaling pathway

Concerning genes involved in the Wnt signaling pathway, over expression of genes encoding negative regulators of this canonical pathway was observed in the NPC whereas genes modulated in MPC cells rather reflected its activation (Figure 4A and 4B). Indeed, Wnt inhibitors including secreted antagonists such as DKK1, SFRP2 and FRZB were all over expressed specifically in NPC. In addition, genes encoding for Wnt ligands such as WNT2B, reported to be a repressor of the canonical pathway, appeared to be specifically up-regulated in NPC cells whereas WNT5A and WNT5B, two non canonical ligands, were found to be modulated in the two types of progenitors. For the Wnt Receptors, notably Frizzled proteins, FZD3 and FZD5 were found respectively up or not regulated in neural progenitors whereas they were both switched off in mesenchymal progenitors. Concerning transcriptional regulators involved downstream of Wnt signalling pathways, genes involved in the repression of the β-catenin complex were found to be up-regulated only in NPC such as the SOX transcription factor SOX3 but also CTNNBIP1, a gene encoding a small soluble inhibitory protein also termed ICAT (Inhibitory of beta-catenin and TCF) which prevents the interaction of β-catenin with different binding partners including LEF1. In addition, the gene encoding for the transcription repressor TLE4, a member of the Groucho family, was also found up-regulated only in the NPC cells. Finally, when looking for the expression of genes known to be controlled directly downstream of the canonical β-catenin pathways, some genes, such as DCT, POU3F2 and NRCAM, controlled downstream of the complex containing LEF1.
appeared to be induced in NPC cells whereas no modulation of these genes was observed in the MPC cells. On the contrary, genes encoding important Wnt-induced mesenchymal markers such as FOSL1, JUN, PPARδ, CD44 were all up-regulated in the MPC whereas the expression of these genes was not modulated in the NPC. To confirm these results, expression modulations for several genes, WNT2B, FRZB, SFRP2, LEF1, involved in Wnt signaling pathway were confirmed by Quantitative PCR using new biological samples in triplicate from three independent differentiation experiment. Results showed that they were found to be up regulated in NPC and not modulated or down-regulated in MPC compared to hES (Figure 4C).

**TGFβ/Activin/BMP signalling pathway**

Among the genes involved in BMP/TGFβ signaling pathways (Figure 5A and 5B), BMP7 expression was up regulated during the differentiation of hESC toward NPC, whereas it was switched off during their differentiation toward MPC. Interestingly, we also found up-regulation of FST gene in MPC encoding follistatin, an inhibitor of BMP pathway (Figure 5C). In contrast, the up regulation of INHBA (inhibin beta A, also termed activin A), which encoded a strong inducer of mesendoderm was overexpressed only in MPC (Figure 5D).

Differences between others modulated genes encoding for TGFβ ligands were also found such as up-regulation of TGBF1 specifically in MPC. Concerning downstream transcriptional factors target, we found an up-regulation of SMAD3 in both NPC and MPC cells respectively whereas SMAD5 was found slightly modulated only in NPC.

At last, in order to confirm expression modulation of genes specifically involved in one of these signaling pathways either in neural or mesenchymal progenitors, quantitative PCR data were carried out from new independent biological samples (Figure S3).

**DISCUSSION**
The principal result of this study is the comparison of gene expression profiles in two homogenous populations of neural and mesenchymal of progenitors by subtractive gene expression profiling during early differentiation in hES toward either neural or mesodermal commitment. Genes whose expressions were regulated in opposite directions might be of particular interest in molecular processes involved in the alternative cell fate decisions. Using a comparative strategy, we identified actors specific for each lineage which play an alternative role at the level of epigenetic modifications, implication of morphogens and through major developmental signalling pathways.

The integration of all these data allowed the construction of a global comprehensive developmental path between neurectoderm and mesoderm (Figure 6).

**Specific gene expression pattern associated with each precursor cell**

Transcriptional factors found to be specifically highly up-regulated in NPC included *LHX2, PAX6, SIX3, SIX6, SOX1* and *FOXG1B*. This gene signature indicated that our neural progenitors exhibited a pattern closed to early neural progenitors appearing first in neural plate and/or neural tube in mouse and human during normal development *in vivo*. Unlike late neural progenitors, these cells expressed markers representative of the anterior region of the mouse developing brain including *FOXG1, EMX2 or OTX2* and markers of dorsal region such as *PAX3 or PAX6*. This “anterior” pattern was associated to a broad capacity of differentiation into various types of neurons and to glial cells in response to appropriate developmental clues. Thus, the present study presented for the first time a picture of gene expression network specifically associated to the neural developmental pathway of these progenitors.

Mesenchymal progenitors derived from hES cells appeared to be the first type of progenitors exhibiting a mesodermal phenotype that can be obtained almost homogeneously [18].
Identification in this study of genes specifically up-regulated in MPC highlighted potential factors that may play a role in the mesoderm formation including *TWIST1*. This gene is expressed in presumptive mesoderm and in invaginating cells in the ventral region of fruit fly embryos and functions in a signaling cascade to initiate mesodermal development during gastrulation in multicellular organisms ranging from Drosophila to vertebrates [24-26].

*TWIST* plays a central role in dorsoventral patterning, which is essential for multiple steps of mesoderm development in Drosophila [27,28]. Another interesting gene includes *WWTR1/TAZ* which was known to control the mechanism of self-renewal through controlling Smads nucleo-cytoplasmic shuttling [29-31] and thus may contributing to the cell fate choice by controlling mesodermal genes. Among other genes that may represent important developmental nodes, *RUNX2, SQSTM1* that are both involved in skeletal development were highlighted suggesting that early mesodermal progenitors acquire a developmental context that enhances osteogenesis.

### Epigenetic modification signatures

The transcriptional signature also decipher the role of another type of developmental regulation that concerns genes involved in epigenetic modifications.

Among genes that are differentially expressed during neural or mesoderm differentiation, genes encoding helicases that function to open chromatin to enhance transcription in the SWI/SNF DNA chromatin remodeling complex family including *SMARCC1* and *SMARCE1*, were found specifically up-regulated in the neural progenitors and may interact with proteins encoded by other specific genes such as *ARID2* and *ARID1B* [32,33]. These proteins may play a role in enhancing differentiation by coupling gene repression with global and local changes in chromatin structure [34]. In mesenchymal precursors, specific up-regulation of *SMARCA2*
was observed, that has been described to be specific of mesoderm in early post-implantation mouse embryos [35].

**Morphogen implications**

Another aspect of the transcriptional signatures concerns morphogens that act through signalling pathways. *BMP7* was found to be up-regulated in neural progenitors and therefore these cells may themselves produce morphogens that contribute to control downstream genes involved in neural development including *ZIC1* [36] and *GLI3* [37]. In MPC, the up-regulation of *INHBA* encoding the beta A subunit that forms a homodimer named Activin-A was reported to be one of the most important mesodermal morphogens in classical developmental models including *Xenopus laevis* [38]. More recently, Willems and Leyns described that Activin A supported self-renewal of hES and directed the nascent mesoderm toward axial mesoderm and mesendoderm [39]. This increase also coincides with up-regulation of the activin receptor type 1, *ACVR1*. Inversely, inhibition of Activin/Nodal signalling promotes specification of hES into neurectoderm [40]. In this context, it is interesting to notify that the *follistatin*, encoding a protein that antagonizes activin [41,42] and BMP signalling pathway in Xenopus embryo [43] is specifically up-regulated in NPC. This might suggest that follistatin may contribute to induce neural differentiation of hES *in vitro* by antagonising the different pathways (BMPs, Activin and TGFβ). Indeed, currently, the most efficient protocol allowing hES differentiation toward neurectoderm uses Noggin (an inhibitor of BMPs) and SB43542 (an artificial molecule inhibiting Activin and TGFβ pathways) [44,45]

Altogether, these data suggest that once the mesenchymal phenotype has been acquired, these precursors may maintain a mesodermal identity by producing themselves important
morphogens such as Activin A and inversely the neurectodermal phenotype may be maintained by producing follistatin that prevents activation of activin pathways.

**Alternative pathway responses**

Wnt and Notch pathway responses occurred during both neural and mesenchymal differentiation. Although discussed, different studies described a “negative” effect of WNT/β-catenin signalling pathways onto the neural induction process *in vivo* [46] and *in vitro* in mouse ES [47,48]. Indeed, an up-regulation of expression of some modulators/inhibitors of the Wnt signalling was observed in NPC (but not in MPC) including *SFRP2*, identified elsewhere to enhance neural differentiation [47] but also *FRZB*, another Wnt inhibitor found expressed in neural plate and neural tube in overlapping areas like SFRP2 during chick development [49]. During and just after gastrulation, the Wnt pathway was also involved as an inducer of primary mesenchymal cells but specific genes involved in this process are still unknown or elusive due to differences between species. In this study, we found that gene expression modulations of Wnt pathway members in mesenchymal cells but not in neural cells including *DKK3* which is both temporally and spatially regulated [50] may play a role in mesoderm formation in humans has been demonstrated in *Xenopus laevis* [51]. Additionally, a distinct profile of genes downstream of the Wnt signalling pathway was also observed. Neural progenitors did not express numerous genes known to be controlled by *CTNNB1* whereas they were expressed in MPC. In contrast, a possible mechanism would be due to the expression of the inhibitor of the interaction of β-catenin with *LEF1*. Thus, *LEF1* may interact with other proteins and control a neural gene network downstream. Concerning the Notch signaling pathway, the central role of NOTCH1 specific pathway already described was confirmed as an important path for the maintenance of the neural progenitor state [52,53] whereas the down-regulation of NOTCH1 was already reported to enhance the differentiation of mesodermal cardiogenic progeny [32]. Here, *DLL1*, one of the NOTCH1 ligands was
found highly up-regulated in NPC and may contribute to induce *HES1* and *HES5* transcription factors acting by their dominant effect on neurogenic transcription factors and therefore to delay the differentiation of neural precursors in mature neurons maintaining a “neural precursor state”. Conversely, *DLL1* was down-regulated in MPC suggesting a process that closely controls this gene during the transition toward either neural or mesoderm identity.

Some genes connected to Notch signalling were also described that may support central interactions in the global neural gene network. For example, *SOX10* that interacts with *PAX3* may reflect some features of neural crest development as demonstrated in cells surrounding neural rosettes in culture [54] and in the neural crest origin of the Waardenburg syndrome in humans [55]. WNT inhibition pathways associated with NOTCH1 activation may be involved in expression of *LEF1* specific expression contributing to an explanation of the specific regulation of downstream neural genes such as *NRCAM*, *DCT* and *POU3F2*. Concerning the acquisition of the mesenchymal phenotype, our data suggested that the differentiation involved a developmental process mimicking the TGFβ1-induced epithelial-mesenchymal transition (EMT) process. Numerous genes involved downstream of TGFbeta1 signalling pathways which have already been described as major contributors of the EMT type 1 process were found to be up-regulated [56,57]. During embryonic development, the EMT is a crucial cellular process whereby adherent cells dissolve their intercellular contacts, organize their motility apparatus, and move to new locations. As observed *in vivo* during the EMT, during mesenchymal differentiation of hES, we found the loss of epithelial cell adhesion markers including E-box genes including E-cadherin [CDH1] but also claudin [CLDN3/6/10/23], occludin [OCLN] and loss of some polarity genes such as Discs Large (DLG3/7) [58]. Crumbs homolog3 (Crb3) [59] and conversely the acquisition of mesenchymal markers including Fibronectin [FN1], Vimentin [VIM] and thrombospondin-1 [THBS1]. It can be postulated that TGFβ1 signalling may reflect in part the biology of the mesenchymal
progenitors. Moreover, SIX1, a developmental gene encoding for a homeodomain transcription factor was the most up-regulated transcription regulator in MPC. It is known to cooperate with TGFβ and to increase the downstream EMT-induced TGFβ signal leading to the acquisition and/or maintenance of the stem cell-like phenotype accompanying EMT [60,61].

In conclusion, based on the production of precursor cell populations at near homogeneity, our data describing modulation of gene expression should contribute to a better comprehension of gene regulations involved in fate choices during differentiation of embryonic stem cells.

ACKNOWLEDGEMENTS:

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AUTHORS INFORMATIONS

The gene expression data have been deposited in GEO Data Bank with the accession number GSE8590.
LEGENDS

**Figure 1**: Global gene expression patterns analysis for the hES cells (ES) and the two precursor cells, NPC and MPC derived from the same hES cells. (A) Schematic representation of the experimental procedure. (B) Correlation plots between all arrays of the experiment hybridized with ES, NPC and MPC mRNAs (n=6 per condition). Correlation was normalised in a scale from $r^2=0.79$ (green) to $r^2=1$ (red). (C) Principal component analysis (PCA) of the three cell populations representative of intra-samples variability across whole chip for each developmental stage. Each array is represented as a colored square in a two-dimensional reference. ES are represented as red squares; MPC as green squares and NPC as blue squares. (D) Genes were selected as differentially expressed either between NPC vs hES or MPC compared to hES for a threshold of FC (Fold change) >2 and a corrected p-value $\alpha<0.05$. The lists of modulated genes in all the conditions were crossed by Venn diagrams.

**Figure 2**: Global gene networks. Networks were constructed using the Ingenuity software based on expression relationships described in the literature. Modulated genes are represented as a box with its gene symbol inside. (A) Global neural gene network and (B) global mesenchymal gene network. Genes in red were up-regulated and genes in green were down-regulated in NPC or MPC compared to hES. Colour intensities of genes were correlated to fold change intensities.

**Figure 3**: Comparison of gene expression modulations within Notch signalling pathway either in NPC or in MPC. Modulated genes are represented as a box with its gene symbol inside. Genes up-regulated by comparison with hES are in red and those down-regulated in green. Colour intensities of genes were correlated to fold change intensities. The following
symbol (▲) indicated a relation of “inhibition” and (▼) a relation of “activation” between genes in the path. (A) Genes modulated between NPC and hES (B) Genes modulated between MPC and hES. (C) Differential gene expression measured by quantitative RT-PCR in hES (ES), MPC, NPC. Results are presented as relative expression level compared to hES cells considered arbitrarily as 1 from 3 independent samples. (t-student, * p<0.05).

**Figure 4**: Comparison of gene expression modulations within canonical Wnt/β catenin pathway either in NPC or in MPC. Same legend as described in Figure 3. (A) Genes specifically modulated between NPC and hES (B) Genes modulated between MPC and hES. (C) Differential gene expression measured by quantitative RT-PCR in hES (ES), MPC, NPC. Results are presented as relative expression level compared to hES cells considered arbitrarily as 1 from 3 independent samples. (t-student, *p<0.05; **p<0.01; ***p<0.001; ns statistically not significant).

**Figure 5**: Comparison of gene expression modulations within TGF Beta/Activin/BMP signalling pathway either in NPC or in MPC. Same legend as described in Figure 3. (A) Genes specifically modulated between NPC and hES (B) Genes modulated between MPC and hES. Differential expression measured by quantitative RT-PCR for (C) Inhibin BetaA, INHBA (Activin A) and (D) FST, Follistatin. (t-student, * p<0.05; ns statistically not significant).

**Figure 6**: Comprehensive developmental path model based on our gene expression data which may be controlled the hES cell fate decision toward either neural or mesodermal fates.
REFERENCES


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216x249mm (150 x 150 DPI)
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Supplementary Figure legends

**Figure S1 : Differentiation and characterization of NPC.** (A) hES cells were cultured under appropriate conditions until DIV21 to obtain neural rosettes. (B) NCAM-positive cells were isolated by cell sorting. (C) Gene expression patterns of the NCAM positive cells were explored by RT-PCR. Cells do not express undifferentiated markers such as *NANOG* or glial makers such as *GFAP* whereas the induction of some expected neur ectodermic markers was observed including *NCAM-1; PAX6; SOX1; OTX2*. No change in the expression of *NES* [Nestin] was observed. Immunostaining revealed that NCAM-positive cells widely expressed NESTIN (D), NCAM, SOX1 (E) and PAX6 (F). Functional characterization of these cells demonstrated their ability to generate both neurons and astrocytes under classical differentiating conditions as highlighted by positive βIII-tubulin (G) and GFAP positive staining (H) respectively.

**Figure S2 : Differentiation and characterization of MPC cells.** (A) hES cells were cultured on gelatin in appropriate conditions until DIV28 to obtain cells with a fibroblast-like morphology. (B) Phenotyping was performed by FACS as described on material and methods. (C) Immunocytochemistry of two mesodermal markers Stro-1 (left, red) and αSMA (right, green) counterstained with DAPI (nuclei in blue). (D) Capacity of MPC to differentiate toward either osteoblasts or adipocytes as described on material and methods.

**Figure S3 : Validation by Q-PCR of key genes identified to be modulated in the transcriptome experiment.** (A) Genes specifically modulated between NPC and hES (B) Genes specifically modulated between MPC and hES. Results are presented as relative expression level compared to hES cells considered arbitrarily as 1 from 3 independent samples. (t-student, *p<0,05; **p<0,01; ***p<0,001; ns statistically not significant).
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181x246mm (150 x 150 DPI)
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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

Stem Cells and Development

Table S2: Genes overexpressed in NPC compared to hES (Fold Change > 2; α < 0.05)

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Table S2: Genes overexpressed in NPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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**Table S2: Genes overexpressed in NPC compared to HES (Fold Change > 2, α < 0.05)**

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.
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Table S2: Genes overexpressed in NPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Global transcriptomic analysis of several cell lines and datasets with a focus on the developmental stages of neural and mesenchymal progenitors derived from human embryonic stem cells is presented. The data is used to identify genes that are differentially expressed between these stages and to provide insights into the underlying molecular mechanisms. Notably, the analysis reveals a number of genes that are overexpressed in NPCs compared to NES, including WNT5B, WNT5A, WNT2B, and WHSC1. These genes are involved in various cellular processes and have been implicated in the regulation of neural development. The study also highlights the importance of transcriptional profiling in understanding the complex regulatory networks that govern cell fate determination in embryonic stem cells.
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### Table S3: Genes downregulated in NPC compared to hES (Fold Change > 2; α < 0.05)

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Table S3: Genes downregulated in NPC compared to hES (Fold Change > 2; α < 0.05)

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Table S3: Genes downregulated in NPC compared to hES (Fold Change > 2; α < 0.05)

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Table S3 : Genes downregulated in NPC compared to hES (Fold Change > 2; a < 0.05)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.
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Table S4: Genes overexpressed in MPC compared to hES (Fold Change > 2; \(a < 0.05\))

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Table S4: Genes overexpressed in MPC compared to hES (Fold Change > 2; a < 0.05)

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Table S4: Genes overexpressed in MPC compared to hES (Fold Change > 2; a < 0.05)

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Table S4: Genes overexpressed in MPC compared to hES (Fold Change > 2; a < 0.05)

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Table S4: Genes overexpressed in MPC compared to hES (Fold Change > 2; a < 0.05)

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

<p>| Table 55: Genes downregulated in MPC compared to hES (Fold Change &gt; 2; ( p &lt; 0.05 )) |
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| BYSL | 705 | chr6 |
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| C10orf95 | 54808 // 79946 | chr10 |
| C11orf1 | 64776 | chr11 |
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| C11orf31 | 280636 | chr11 |
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| C13orf3 | 221150 | chr13 |
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| C14orf1 | 11161 | chr14 |
| C14orf104 | 55172 | chr14 |
| C14orf106 | 55329 | chr14 |
| C14orf109 | 28175 | chr14 |
| C14orf115 | 55237 | chr14 |
| C14orf120 | 25983 | chr14 |
| C14orf122 | 51016 | chr14 |
| C14orf130 | 55148 | chr14 |
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| C14orf145 | 145508 | chr14 |
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| C14orf156 | 81892 | chr14 |
| C14orf159 | 80017 | chr14 |
| C14orf169 | 79697 | chr14 |
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| C18orf18 | 147525 | chr18 |
| C18orf19 | 125228 | chr18 |
| C18orf22 | 79863 | chr18 |
| C18orf37 | 125476 | chr18 |
| C18orf54 | 162681 | chr18 |
| C18orf55 | 29090 | chr18 |
| C18orf9 | 79959 | chr18 |
| C19orf32 | 92840 | chr19 |
| C19orf33 | 64073 | chr19 |
| C19orf7 | 23211 | chr19 |
| C19orf71 | 56913 | chr19 |
| C11orf104 | 284618 | chr1 |
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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( α < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \(p < 0.05\))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

| FLJ21168 | 80143 | hypothetical protein FLJ21168 // hypothetical protein FLJ21168 | 2.05 | chr1 |
| FLJ21816 | 79728 | hypothetical protein FLJ21816 | 3.96 | chr16 |
| FLJ21839 | 60509 | hypothetical protein FLJ21839 | 2.65 | chr2 |
| FLJ21865 | 64772 | endo-beta-N-acetylglucosaminidase | 2.23 | chr17 |
| FLJ21901 | 79675 | hypothetical protein FLJ21901 | 4.79 | chr2 |
| FLJ21924 | 79832 | hypothetical protein FLJ21924 | 6.81 | chr11 |
| FLJ21945 | 80304 | hypothetical protein FLJ21945 | 3.73 | chr2 |
| FLJ21963 | 79611 | FLJ21963 protein | 7.45 | chr12 |
| FLJ22104 | 65084 | hypothetical protein FLJ22104 | 2.64 | chr11 |
| FLJ22318 | 64777 | hypothetical protein FLJ22318 | 2.99 | chr5 |
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| FLJ22624 | 79866 | FLJ22624 protein | 5.99 | chr13 |
| FLJ22662 | 79887 | hypothetical protein FLJ22662 | 26.69 | chr12 |
| FLJ22795 | 80154 | hypothetical protein FLJ22795 | 2.05 | chr15 |
| FLJ23342 | 79684 | Hypothetical protein FLJ23342 | 3.02 | chr11 |
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| FLJ23556 | 79938 | hypothetical protein FLJ23556 | 6.18 | chr10 |
| FLJ23861 | 151050 | hypothetical protein FLJ23861 | 2.76 | chr2 |
| FLJ25006 | 124923 | hypothetical protein FLJ25006 | 6.04 | chr17 |
| FLJ25076 | 134111 | similar to CG4502-PA | 4.90 | chr5 |
| FLJ25222 | 374666 | CXXorf1-related protein | 2.71 | chr2 |
| FLJ25416 | 220042 | hypothetical protein FLJ25416 | 9.94 | chr11 |
| FLJ25778 | 254048 | Hypothetical protein FLJ25778 | 4.22 | chr7 |
| FLJ25967 | 440823 | hypothetical gene supported by AK098833 | 27.57 | chr22 |
| FLJ26175 | 386586 | FLJ26175 protein | 2.23 | chr19 |
| FLJ27354 | 400761 | hypothetical gene supported by AK130864 | 14.74 | chr1 |
| FLJ30046 | 122660 | hypothetical protein FLJ30046 | 15.55 | chr13 |
| FLJ30428 | 150519 | similar to hypothetical protein A230046P18; cDNA sequence BC055759 | 8.21 | chr2 |
| FLJ30656 | 124801 | hypothetical protein FLJ30656 | 2.81 | chr8 |
| FLJ30707 | 220108 | hypothetical protein FLJ30707 | 12.56 | chr13 |
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| FLJ31978 | 144423 | hypothetical protein FLJ31978 | 2.16 | chr12 |
| FLJ32009 | 220001 | hypothetical protein FLJ32009 | 2.44 | chr11 |
| FLJ32363 | 375444 | FLJ32363 protein | 3.57 | chr5 |
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| FLJ33318 | 162461 | hypothetical protein FLJ33318 | 2.43 | chr17 |
| FLJ34208 | 401106 | Hypothetical gene supported by AK091527 | 2.22 | chr3 |
| FLJ35119 | 126074 | hypothetical protein FLJ35119 | 3.15 | chr19 |
| FLJ35348 | 266655 | FLJ35348 | 3.46 | chr9 |
| FLJ35801 | 150291 | hypothetical protein FLJ35801 | 3.03 | chr22 |
| FLJ35934 | 400579 // 400569 | hypothetical gene supported by AK093253 | 2.15 | chr17 |
| FLJ36954 | 166968 | hypothetical protein FLJ36954 | 2.74 | chr5 |
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| FLJ37478 | 339983 | hypothetical protein LOC339983 | 2.89 | chr4 |
| FLJ37953 | 129450 | hypothetical protein FLJ37953 | 2.11 | chr2 |
| FLJ38426 | 283742 | hypothetical protein FLJ38426 | 2.24 | chr15 |
| FLJ38973 | 205327 | hypothetical protein FLJ38973 | 4.57 | chr2 |
| FLJ38991 | 285521 | mitochondrial COX18 | 2.14 | chr4 |
| FLJ39616 | 51275 | apoptosis-related protein PNAS-1 | 2.64 | chr12 |
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| FLJ40092 | 401196 | FLJ40092 protein | 6.32 | chr5 |
| FLJ40142 | 400079 | FLJ40142 protein | 2.30 | chr12 |
| FLJ40432 | 151195 | hypothetical protein FLJ40432 | 2.54 | chr2 |
| FLJ40869 | 348654 | hypothetical protein FLJ40869 | 2.14 | chr2 |
| FLJ41131 | 284325 | FLJ41131 protein | 2.32 | chr19 |
| FLJ44186 | 346689 | FLJ44186 protein | 5.11 | chr7 |
| FLJ46072 | 286077 | FLJ46072 protein | 7.62 | chr8 |
| FLJ46419 | 388507 | FLJ46419 protein | 11.93 | chr19 |
| FLJ90024 | 129303 | fastigial-inducible integral membrane protein TM6P1 | 2.01 | chr2 |
| FLJ90036 | 255403 | hypothetical protein FLJ90036 | 4.15 | chr4 |
| FLJ90086 | 389389 | Similar to A1614523 protein | 4.72 | chr6 |
| FLJ90231 | 283176 | hypothetical protein FLJ90231 | 18.33 | chr11 |

Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; P < 0.05)
Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; p < 0.05)

<p>| FLJ90652 | 283899 | hypothetical protein FLJ90652 | 2.34 | chr16 |
| FLT1 | 2321 | Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor) | 3.96 | chr13 |
| FLVCR1 | 28982 | feline leukemia virus subgroup C cellular receptor | 15.20 | chr1 |
| FN5 | 56935 | FNS protein | 2.60 | chr11 |
| FNBP1L | 54874 | Fornin binding protein 1-like | 4.18 | chr1 |
| FNBP3 | 55665 | formin binding protein 3 | 2.53 | chr2 |
| FOS | 2353 | v-fos FB2 murine osteosarcoma viral oncogene homolog | 3.11 | chr14 |
| FOXA3 | 3171 | forkhead box A3 | 29.89 | chr19 |
| FOXD3 | 27022 | Forkhead box D3 | 4.63 | chr1 |
| FOXH1 | 8928 | Forkhead box H1 | 16.42 | chr8 |
| FOXM1 | 2305 | forkhead box M1 | 5.94 | chr12 |
| FOXO1A | 2308 | forkhead box O1A (rhabdomysarcoma) | 10.79 | chr13 |
| FOXO6 | 343552 | forkhead box protein O6 | 6.29 | chr1 |
| FRA2 | 27315 | FGF receptor activating protein 1 | 4.79 | chr9 |
| FRA3 | 80144 | Fraser syndrome 1 | 47.12 | chr4 |
| FRA1T | 10023 | frequently rearranged in advanced T-cell lymphomas | 4.48 | chr10 |
| FRA2T | 23401 | frequently rearranged in advanced T-cell lymphomas 2 | 28.64 | chr10 |
| FREM2 | 341640 | FRAS1 related extracellular matrix protein 2 | 17.37 | chr13 |
| FSD1 | 79187 | fibronectin type III and SPRY domain containing 1 | 3.17 | chr19 |
| FSHPR1 | 2491 | FSH primary response (LRPR1 homolog, rat) 1 | 2.45 | chrX |
| FTSJ2 | 29960 | Ftsj homolog 2 (E. coli) | 2.61 | chr7 |
| FTSJ3 | 117246 | Ftsj homolog 3 (E. coli) | 2.03 | chr17 |
| FUBP1 | 8880 | Far upstream element (FUSE) binding protein 1 | 3.27 | chr1 |
| FUS | 2521 | fusion (involved in t(12;16) in malignant liposarcoma) | 6.51 | chr16 |
| FUSP1 | 10772 | FUS interacting protein (serine/arginine-rich) 1 | 2.83 | chr1_random |
| FUT1 | 2523 | fucosyltransferase 1 (galactoside 2-alpha-L-fucosyltransferase) | 3.99 | chr12 |
| FUT10 | 84750 | fucosyltransferase 10 (alpha (1,3) fucosyltransferase) | 2.78 | chr8 |
| FUT4 | 2585 | fucosyltransferase 4 (alpha (1,3) fucosyltransferase, myeloid-specific) | 2.77 | chr1 |
| FXN | 2395 | frataxin | 3.59 | chr9 |
| FXR1 | 8087 | fragile X mental retardation, autosomal homolog 1 | 3.89 | chr3 |
| FXYD6 | 53826 | FXYD domain containing ion transport regulator 6 | 9.99 | chr11 |
| FZD3 | 7976 | frizzled homolog 3 (Drosophila) | 11.16 | chr8 |
| FZD5 | 7855 | frizzled homolog 5 (Drosophila) | 3.52 | chr2 |
| GJBP1 | 10146 | Ras-GTAP-activating protein SH3-domain-binding protein | 3.99 | chr5 |
| GJBP2 | 9908 | Ras-GTAP activating protein SH3 domain-binding protein 2 | 3.32 | chr4 |
| GAB1 | 2549 | GRB2-associated binding protein 1 | 2.61 | chr4 |
| GABBR1 | 2550 | gamma-aminobutyric acid (GABA) B receptor, 1 | 2.10 | chr6 |
| GABPB1 | 2553 | GA binding protein transcription factor, beta subunit 2 | 3.21 | chr15 |
| GABRB3 | 2562 | gamma-aminobutyric acid (GABA) A receptor, beta 3 | 100.50 | chr15 |
| GABRB3 | 1653 /// 2562 | Gamma-aminobutyric acid (GABA) A receptor, beta 3 /// DEAD (Asp-Glu-Ala-Asp) domain containing protein | 55.60 | chr15 |
| GADD45GIP1 | 90480 | growth arrest and DNA-damage-inducible, gamma interacting protein 1 | 2.63 | chr19 |
| GAJ | 84057 | GAJ protein | 13.42 | chr4 |
| GAL | 51083 | galalin | 53.56 | chr11 |
| GALNAC4S-6 | 51363 | B cell RAG associated protein | 9.57 | chr10 |
| GALNT12 | 79695 | UDP-N-acetyl-alpha-D-galactosamine polyepitope N-acetylgalactosaminyltransferase | 13.20 | chr9 |
| GALNT13 | 114805 | UDP-N-acetyl-alpha-D-galactosamine polyepitope N-acetylgalactosaminyltransferase | 3.78 | chr2 |
| GALNT3 | 2591 | UDP-N-acetyl-alpha-D-galactosamine polyepitope N-acetylgalactosaminyltransferase | 12.62 | chr2 |
| GAP43 | 2596 | growth associated protein 43 | 12.36 | chr3 |
| GAPDH | 26330 | Glyceraldehyde-3-phosphate dehydrogenase, spastomagenic | 4.10 | chr19 |
| GARNL1 | 253959 | GTPase activating Rap/RanGAP domain-like 1 | 2.76 | chr9 |
| GARNL4 | 23108 | GTPase activating Rap/RanGAP domain-like 4 | 4.66 | chr17 |
| GART | 2618 | Phosphoribosylglycaminide formyltransferase, phosphoribosylglycinamin synthase | 5.03 | chr21 |
| GATA1D | 57798 | GATA zinc finger domain containing 1 | 2.02 | chr7 |
| GATA2D | 54815 | GATA zinc finger domain containing 2A | 2.63 | chr19 |
| GATA2B | 57459 | GATA zinc finger domain containing 2B | 2.09 | chr1 |
| GATM | 2628 | glycine amidotransferase (L-arginine:glycine amidotransferase) | 3.72 | chr15 |
| GBA2 | 57704 | Glucosidase, beta (bile acid) 2 | 3.20 | chr9 |
| GCA | 25801 | grancalcin, EF-hand calcium binding protein /// grancalcin, EF-hand calcium binding protein | 9.28 | chr2 |
| GCDH | 2639 | glutaryl-Coenzyme A dehydrogenase | 2.72 | chr19 |
| GCH1 | 2643 | GTP cyclohydrolase 1 (dopa-responsive dystonia) | 3.95 | chr14 |
| GCHFR | 2644 | GTP cyclohydrolase I feedback regulator | 3.42 | chr15 |
| GCL | 64395 | Germ cell-less homolog 1 (Drosophila) | 2.43 | chr2 |
| GCLC | 2729 | glutamate-cysteine ligase, catalytic subunit | 4.53 | chr6 |
| GCT1 | 2650 | glucosaminyl (N-acetyl) transferase 1, core 2 (beta-N-acetylglucosaminyltransferase) | 2.73 | chr9 |
| GGT2 | 2651 | glucosaminyl (N-acetyl) transferase 2, 1-branching enzyme | 51.06 | chr6 |
| GCSH | 2653 | glycine cleavage system protein H (aminomethyl carrier) | 4.63 | chr5 |
| GDAP1 | 54332 | Ganglioside-induced differentiation-associated protein 1 | 10.97 | chr8 |</p>
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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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## Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))
Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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| KIAA1815 | KIAA1815 | 3.86 | chr9 |
| KIAA1906 | KIAA1906 protein | 7.00 | chr12 |
| KIAA1909 | KIAA1909 protein | 6.79 | chr5 |
| KIAA1935 | KIAA1935 protein | 6.69 | chr5 |
| KIAA1944 | KIAA1944 protein | 2.84 | chr12 |
| KIAA1958 | KIAA1958 | 18.10 | chr9 |
| KIAA1970 | KIAA1970 protein | 3.99 | chr16 |
| KIAA1982 | KIAA1982 protein | 5.40 | chr4 |
| KIAA2010 | KIAA2010 | 2.17 | chr14 |
| KIBRA | KIBRA protein | 4.01 | chr5 |
| KIF11 | kinesin family member 11 | 6.99 | chr10 |
| KIF14 | kinesin family member 14 | 7.32 | chr1 |
| KIF15 | kinesin family member 15 | 11.53 | chr3 |
| KIF18A | kinesin family member 18A // kinesin family member 18A | 2.36 | chr11 |
| KIF1A | kinesin family member 1A | 40.11 | chr2 |
| KIF1B | kinesin family member 1B | 5.00 | chr1 |
| KIF20A | kinesin family member 20A | 8.23 | chr5 |
| KIF21A | kinesin family member 21A | 6.33 | chr12 |
| KIF22 | kinesin family member 22 | 10.39 | chr16 |
| KIF23 | kinesin family member 23 | 3.68 | chr15 |
| KIF26A | kinesin family member 26A | 4.00 | chr14 |
| KIF2C | kinesin family member 2C | 6.72 | chr1 |
| KIF4A | kinesin family member 4A | 5.74 | chr5 |
| KIF5A | Kinesin family member 5A | 18.02 | chr12 |
| KIF5C | Kinesin family member 5C | 147.77 | chr2 |
| KIF9 | kinesin family member 9 | 2.26 | chr3 |
| KIFC1 | kinesin family member C1 | 2.87 | chr6 |
| KIT | \( \gamma \)-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog | 12.41 | chr4 |
| KLF4 | Kruppel-like factor 4 (gut) | 6.08 | chr9 |
| KLF8 | Kruppel-like factor 8 | 6.52 | chrX |
| KLHDC4 | Kelch domain containing 4 | 2.57 | chr16 |
| KLHL12 | Kelch-like 12 (Drosophila) | 2.08 | chr22 |
| KLHL13 | Kelch-like 13 (Drosophila) | 3.96 | chrX |
| KLHL23 | Kelch-like 23 (Drosophila) | 8.07 | chr2 |
| KLHL3 | Kelch-like 3 (Drosophila) | 4.86 | chr5 |
| KLHL7 | Kelch-like 7 (Drosophila) | 7.93 | chr7 |
| KLKB1 | Kallikrein B, plasma (Fletcher factor) 1 | 10.84 | chr4 |
| KNTC1 | kinetochore associated 1 | 6.70 | chr12 |
| KNTC2 | kinetochore associated 2 | 6.21 | chr18 |
| KPNA2 | karyopherin alpha 2 (RAG cohort 1, importin alpha 1) // karyopherin alpha 2 (RAG cohort 1, importin alpha 1) | 2.94 | chr17 |
| KPNA3 | Karyopherin alpha 3 (importin alpha 4) | 2.83 | chr13 |
| KPNA5 | Karyopherin alpha 5 (importin alpha 6) | 2.01 | chr6 |
| KPNB1 | Karyopherin (importin) beta 1 | 2.02 | chr17 |
| KPTN | Kapartin (actin binding protein) | 3.41 | chr9 |
| KRTAP4-7 | Keratin associated protein 4-7 | 5.40 | chr15 |
| KRTAP53 | Keratinocyte associated protein 3 | 11.82 | chr2 |
| KUB3 | Ku70-binding protein 3 | 2.86 | chr12 |
| L2HGIDH | L-2-hydroxyglutarate dehydrogenase | 3.06 | chr14 |
| LACTB2 | Lactamase, beta 2 | 2.05 | chr8 |
| LAM1 | laminin, alpha 1 | 21.06 | chr18 |
| LAMC3 | laminin, gamma 3 | 2.39 | chr9 |
| LANC1 | Lantibiotic synthetase component C-like 2 (bacterial) | 2.39 | chr7 |
| LAPTM4B | lysosomal associated protein transmembrane 4 beta | 6.03 | chr8 |
| LARP2 | La ribonucleoprotein domain family, member 2 | 3.81 | chr4 |
| LARP4 | La ribonucleoprotein domain family, member 4 | 2.34 | chr12 |
| LARP5 | La ribonucleoprotein domain family, member 5 | 2.44 | chr10 |
| LARS | Leucyl-tRNA synthetase | 2.62 | chr5 |
| LARS2 | Leucyl-tRNA synthetase, mitochondrial | 4.24 | chr3 |
| LAS1L | LAS1-like (S. cerevisiae) // LAS1-like (S. cerevisiae) | 4.72 | chrX |
| LASS1 | LAG1 longevity assurance homolog 1 (S. cerevisiae) | 2.87 | chr19 |
| LASS4 | LAG1 longevity assurance homolog 4 (S. cerevisiae) | 2.08 | chr19 |
| LASS6 | LAG1 longevity assurance homolog 6 (S. cerevisiae) | 3.09 | chr2 |
| LBR | lamin B receptor | 2.94 | chr1 |
| LCHN | LCHN protein | 3.72 | chr7 |
| LCK | lymphocyte-specific protein tyrosine kinase | 37.69 | chr1 |
Stem Cells and Development

Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.
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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \)).

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; p < 0.05).

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2, α < 0.05)

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

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SBK1 388228 SH3-binding domain kinase 1 28.31 chr16
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SDF3B3 23450 splicing factor 3b, subunit 3, 130KDa 2.34 chr16
SFMBT1 51460 Scm-like with four mbd domains 1 5.51 chr3
SFMBT2 57713 Scm-like with four mbd domains 2 2.37 chr10
SFN 2810 straflin 6.90 chr1
SFPQ 6421 Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated) 3.61 chr1
SFRP2 6423 secreted frizzled-related protein 2 113.98 chr4
SFRS1 6426 Splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor) 4.65 chr17
SFRS10 6434 splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila) 3.15 chr3
SFRS12 140890 Splicing factor, arginine/serine-rich 12 2.11 chr5
SFRS15 57466 splicing factor, arginine/serine-rich 15 2.82 chr21
SFRS2 6427 splicing factor, arginine/serine-rich 2 3.19 chr17
Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

<p>| Table S5. Genes downregulated in MPC compared to hES (Fold Change &gt; 2; p &lt; 0.05) |
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| SFRS3                          | 6428            | 3.82            |
| SFRS4                          | 6429            | 2.19            |
| SFRS5                          | 6430            | 2.09            |
| SFRS7                          | 6432            | 6.09            |
| SFT2D1                         | 113402          | 2.23            |
| SXN2                           | 118980          | 3.66            |
| SXNXS                          | 94097           | 2.09            |
| SGEF                           | 26084           | 6.00            |
| SGEI1                          | 6447            | 2.70            |
| SGOL2                          | 151246          | 3.78            |
| STAG                           | 6449            | 2.22            |
| SHGL2                          | 6456            | 4.09            |
| SHGGL3                         | 6457            | 3.72            |
| SHANK2                         | 22941           | 8.11            |
| SHMT1                          | 6470            | 3.69            |
| SNP1                           | 257218          | 3.64            |
| SIAH1                          | 6477            | 2.52            |
| SIAHBP1                        | 22827           | 2.00            |
| SIGIRR                         | 59307           | 3.44            |
| SIL                            | 6491            | 4.52            |
| SILV                           | 6490            | 30.56           |
| SNS3A                          | 25942           | 3.15            |
| SIP1                           | 8487            | 2.35            |
| SIPA1L2                        | 57568           | 3.70            |
| SIRT1                          | 23411           | 6.15            |
| SIRT5                          | 23408           | 2.69            |
| SITPEC                         | 51295           | 2.73            |
| SNA                           | 10572           | 3.49            |
| SKB1                           | 10419           | 2.09            |
| SKIL                           | 6498            | 2.26            |
| SKIV2L2                        | 23517           | 3.94            |
| SKP2                           | 6502            | 8.02            |
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| SLC12A9                        | 56996           | 2.21            |
| SLC15A4                        | 121260          | 2.21            |
| SLC16A1                        | 6566            | 7.32            |
| SLC16A10                       | 117247          | 20.68           |
| SLC16A4                        | 9122            | 6.68            |
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| SLC25A29                       | 112386          | 2.67            |
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| SLC27A2                        | 11001           | 5.76            |
| SLC27A3                        | 11000           | 10.12           |
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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; α < 0.05)

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

### Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; p < 0.05)

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Table S5: Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)


Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( p < 0.05 \))

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Table S5. Genes downregulated in MPC compared to hES (Fold Change > 2; \( \alpha < 0.05 \))

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Global transcriptional profiling of neural and mesenchymal progenitors derived from human embryonic stem cells reveals alternative developmental signaling pathways (doi: 10.1089/scd.2010.0331)

This article has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

<p>| ZNF418 | 147686 | zinc finger protein 418 | 4.65 | (vide) |
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| ZNF423 | 23090 | zinc finger protein 423 | 9.65 | chr16 |
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| ZNF430 | 80264 | zinc finger protein 430 | 2.45 | chr19 |
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| ZNF607 | 84775 | zinc finger protein 607 | 4.26 | chr19 |
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| ZNF677 | 342926 | Zinc finger protein 677 | 4.29 | chr19 |
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| ZNF682 | 91120 | zinc finger protein 682 | 7.89 | chr19 |
| ZNF689 | 115509 | zinc finger protein 689 | 3.65 | chr16 |
| ZNF690 | 146050 | zinc finger protein 690 | 2.46 | chr15 |
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| ZNF694 | 342357 | zinc finger protein 694 | 2.16 | chr16 |
| ZNF700 | 90592 | zinc finger protein 700 | 2.58 | chr19 |
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Additional information:
- Primary neuroblastoma cDNA, clone:Nbla11652
- chromosome 10 open reading frame 58 // chromosome 10 open reading frame 70.9 // reticulin 4 interacting protein 1 // reticulin 4 interacting protein 1
- WW domain containing oxidoreductase
- Fancori anemia, complementation group A // Fancori anemia, complementation group B // proteasome (prosome, macropain) subunit, beta type, 2 // proteasome (prosome, macropain) subunit, beta type, 3
- melanoma cell adhesion molecule // melanoma cell adhesion molecule
- tubulin, beta, 2
- zinc finger protein 146 // zinc finger protein 146
- activating signal cointegrator 1 complex subunit 3-like 1 // activating signal cointegrator 1 complex subunit 3-like 2
- actin related protein 2/3 complex, subunit 5-like // actin related protein 2/3 complex, subunit 5-like
Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change > 2; \( \alpha < 0.05 \))

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### Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change >2 ; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change>2; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change >2; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change > 2; α < 0.05)

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## Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change>2; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change>2; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change>2; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change>2 ; α<0.05)

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Table S6: Genes overexpressed in NPC compared to hES and not modulated in MPC compared to hES (Fold change >2; α<0.05)

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Table S7: Genes overexpressed in NPC compared to hES and down-regulated in MPC compared to hES (FC>2; α<0.05)

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Table S7: Genes overexpressed in NPC compared to hES and down-regulated in MPC compared to hES (FC>2; α<0.05)
<table>
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<tr>
<th>Gene Symbol</th>
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Table S7: Genes overexpressed in NPC compared to hES and down-regulated in MPC compared to hES (FC>2 ; α<0.05)

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Table S8: Genes up-regulated in MPC compared to hES and not modulated in NPC compared to hES (FC>2; α<0.05)

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### Table S9: Genes overexpressed in MPC compared to hES and down-regulated in NPC compared to hES (94 genes FC>2; α<0.05)

<table>
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<tr>
<th>Gene Symbol</th>
<th>Entrez Gene</th>
<th>Gene Title</th>
<th>MPC_up</th>
<th>NPC_down</th>
<th>Chromosome Number (Avadis)</th>
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Table S9: Genes overexpressed in MPC compared to hES and down-regulated in NPC compared to hES (94 genes FC>2; α<0.05)

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<th>Gene Symbol</th>
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**Abbreviations:**
- **NCAM:** neural cell adhesion molecule (NCAM)
- **NPC:** neural progenitor cells
- **hES:** human embryonic stem cells
- **MPC:** mesenchymal progenitor cells
- **FC:** fold change
- **NS:** no significant modulation

The table lists various genes and their fold changes in expression compared to controls, highlighting those with significant modulation. The data sets include NCAM-NPC vs hES and CD73-MPC vs hES, with some genes showing modulation indicated by FC (fold change) values.